RESEARCH HIGHLIGHTS
NATIONAL INSTITUTES OF HEALTH

1958

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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1958

Items of Interest on Research Studies
Conducted and Supported by the Institutes
and Divisions of NIH

As Presented to the
Congress of the United States

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

CONTENTS

Introduction	1
National Cancer Institute	3
National Heart Institute	55
National Institute of Allergy and Infectious Diseases	99
National Institute of Arthritis and Metabolic Diseases	139
National Institute of Dental Research	163
National Institute of Mental Health	177
National Institute of Neurological Diseases and Blindness	217
Clinical Center	245
Division of Biologics Standards	247
Division of General Medical Sciences	255
Division of Research Services	273

Office of Research Information
National Institutes of Health
Public Health Service
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

INTRODUCTION

For several years the National Institutes of Health, main research center of the Public Health Service, has submitted statements supplementing its testimony at congressional hearings on appropriations to the U. S. Department of Health, Education, and Welfare. The following pages comprise the statements called Research Highlights as prepared by the Institutes and Divisions of NIH for hearings on the fiscal 1960 budget.

The compilation is intended to meet requests and administrative needs. It contains representative items selected in light of both scientific importance and public interest. In no sense is it a comprehensive review of NIH activities, nor even necessarily of its most significant results.

The items concern the work of both staff scientists (laboratory and clinical) and research grantees. No attempt has been made to reflect the relative magnitude of the grants program. During the period covered--calendar year 1958--more than two-thirds of NIH funds for research were awarded to investigators in universities, medical schools, and other non-Federal research centers.

The items in this report are based on published work. The scientists' articles appear in a wide range of journals, mainly non-Federal, which are available in medical libraries. Further information, including references to these articles, may be obtained through the Office of Research Information, NIH, or the information office of the Institute concerned.

HIGHLIGHTS OF PROGRESS

IN CANCER RESEARCH

1958

Items of Interest on Research Studies Conducted and Supported by

the National Cancer Institute

The search for new knowledge about cancer is spurred by the conviction that some day the disease will be more effectively controlled by treatment and prevention. Progress toward this goal during 1958 was measured by advances reported in many different scientific disciplines. In virology, pharmacology, epidemiology, and other areas of cancer research, new information was discovered on the causation, characteristics, detection, and treatment of cancer.

New evidence on the causation of animal cancers was found in a study showing that the mouse-tumor agent, which causes many types of cancers in mice, hamsters, and other species, is a virus. Earlier findings showing a statistical association between lung cancer and cigarette smoking were confirmed in a study of 7,000 veterans of World War I, and a similar relationship was observed in a study of a group of 160 women. Development of a quantitative technique for preparing human whole blood so that it can be examined cytologically for the presence of malignant cells was reported. This technique makes possible the study of the significance of circulating tumor cells in patients.

Progress was reported in the development of the cytoanalyzer, which gave accurate results for all the positive or suspicious slides and 40 percent of the negative slides in a test of 1,100 known slides obtained in the cytologic test for uterine cancer. Investigations in chemotherapy resulted in the development of several new drugs that are now in clinical trial.

The items presented in this report were selected as representative of the highlights of progress in research studies conducted and supported by the National Cancer Institute. They include significant advances in many areas of clinical and laboratory investigation. Institute staff scientists are identified by the Laboratory or Branch with which they are connected. Grantees are identified by their

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institution. Only the name of the senior author is usually given.

CAUSATION OF CANCER

FURTHER STUDIES MADE OF MOUSE TUMOR AGENT

An increasingly important area of cancer research is concerned with efforts to learn whether human cancer is caused by viruses. Virus-induced tumors of mice, chickens, and other animals are being intensively studied as model systems by a variety of techniques and methods that may produce important leads in human cancer research.

Dr. Sarah E. Stewart, Laboratory of Biology, and Dr. Bernice E. Eddy, Division of Biologics Standards, reported further results on a tumor-inducing agent that they first described about two years ago.

Drs. Stewart and Eddy reported that the potency of the tumor-inducing agent present in mouse leukemia extracts is increased if it is carried in tissue cultures of minced, mouse embryo cells instead of monkey kidney cells. The increased activity of the agent cultured by the improved technique was shown by a greater frequency in production of certain primary tumors in injected mice; a shortening of the latent period required for appearance of the tumors after inoculation; retention of activity by 5 sublines of the agent through many serial passages in tissue culture; and retention of tumor-inducing activity of the agent at dilutions of 10^{-5} (100,000 fold). A total of 23 different neoplastic lesions was produced in the injected mice.

In another study, Drs. Stewart and Eddy reported that tumors were induced in hamsters by the mouse tumor agent from tissue cultures containing minces or extracts of mouse leukemias.

The tumors occurred at an age when extremely few spontaneous neoplasms have been described in hamsters. The response in hamsters is different from the one following administration of comparable materials to new-born mice. Hamsters develop sarcomas and vascular tumors almost exclusively, whereas mice exhibit a wider variety of neoplasms.

MOUSE TUMOR AGENT HAS BIOLOGICAL AND PHYSICAL PROPERTIES OF A VIRUS

The investigators also reported that the agent possesses certain fundamental properties characteristic of viruses. Tumors were produced in hamsters and mice by tumor extracts that were passed through tissue culture many times. Tumor-producing activity of the agent was

increased by alternate passage through tissue culture and mice or hamsters. The agent is destroyed by heat but not by freezing temperatures. Its size is in the virus range, as demonstrated by the pore size of filters through which it passes. The agent produces neutralizing antibodies in rabbits and is unaffected by antibiotics, which commonly destroy bacteria but not viruses.

Concentration of infected tissue culture fluid or partial purification of the active principle by chemical means accelerates the tumor-inducing ability of the agent. This indicates that the agent is concentrated by these methods and that its activity is related to the size of the dose.

The collaborative study of the agent, now called polyoma virus, originated in observations by Dr. Stewart that injection of new-born mice with cell-free mouse leukemia extracts produced an unusual type of tumor of the parotid (salivary) gland that rarely occurs spontaneously in mice. Injection of new-born mice with cell-free extracts prepared from the parotid gland tumors did not produce tumors.

But when cell suspensions of parotid gland tumor tissue were maintained in tissue culture and the cell-free supernatant fluids injected into new-born mice, the mice developed tumors. All mice that developed such tumors had primary parotid gland tumors; and some also developed tumors of the thymus, adrenal glands, and mammary glands. Similar results were obtained when an extract from a spontaneous mouse leukemia was used.

The observations reported by Drs. Stewart and Eddy, have been confirmed by other investigators. Dr. Edwin A. Mirand, Roswell Park Memorial Institute, reported the production of multiple types of tumors in mice, rats, and hamsters treated with a mouse tumor agent propagated in mouse embryo tissue culture.

IMMUNIZATION PROCEDURE IN HAMSTERS PREVENTS POLYOMA VIRUS TUMORS

In an extension of their research, Drs. Stewart and Eddy reported that the development of tumors induced in hamsters by the polyoma virus can be prevented by an immunization procedure. This involves injecting the young hamsters with a "neutral" mixture containing polyoma virus and specific antibodies against the agent.

Hamsters were injected with the virus-antibody mixture when two days old and again six days later. On challenge with the active virus alone two weeks after the second immunizing injection, 97

percent of the hamsters failed to develop tumors and survived 6 months at which time the experiment was terminated. Sixty-seven percent of the unimmunized control litter mates that received the same virus challenge died with tumors within five months.

ROUS SARCOMA VIRUS
OBSERVED UNDER
ELECTRON MICROSCOPE

In order to relate a causal agent to a disease entity, an important requisite that must be established is the demonstration of a consistent and quantitative relationship between the severity of the disease and the number of particles of the presumed causal agent present. The techniques of electron microscopy, assist in visualizing virus particles directly in the tissues of the host.

Dr. Francois Haguenau, Institut de Recherches sur le Cancer, Villejiuf, France, and Dr. A. J. Dalton and J.B. Moloney, National Cancer Institute Laboratory of Biology have reported results of a collaborative investigation of electron microscopic studies of the Rous virus, which causes sarcoma in chickens. Then sections of samples of tumors induced with high and low doses of Rous sarcoma virus were examined with the electron microscope. Counts were made of the number of particles per cell section. The results of the counts were compared with the potency of extracts of these tumors as determined by bioassay.

Within high-dose groups there was a rough correlation between the number of particles and the potency of extract. These results provide further evidence that these particles represent the agent responsible for the induction of the Rous chicken sarcoma.

ELECTRON MICROSCOPE SHOWS
MOUSE LEUKEMIA VIRUS

Preliminary results of an electron microscope study revealing virus-like particles in leukemia mouse tissues have been reported by Dr. Etienne de Harven and Dr. Charlotte Friend, Sloan-Kettering Institute for Cancer Research. Virus-like particles were observed in about one-fourth of the specimens examined.

The study was done in mice that developed leukemia when they were inoculated with an agent discovered about two years ago by Dr. Friend. Fully grown mice infected with this agent develop signs of leukemia 14 to 21 days after inoculation.

Specimens for the study were prepared from tissues of leukemic mice, including spleen, liver, and bone marrow. The virus-like particles observed under the electron microscope were located inside the cytoplasm of leukemic cells that infiltrated these organs.

The particles, approximately 78 mμ. in diameter, were composed of two concentric spherical shells. They were fundamentally different from any known components of normal cytoplasm, but comparable to structures observed in pathological cells derived from Rous chicken sarcoma, mouse mammary carcinoma, cells infected with influenza virus, and HeLa cells infected with RI-APC virus.

FACTORS OTHER THAN TUMOR AGENT CAUSE MAMMARY TUMORS IN MICE

In addition to viruses, other possible cancer-causing factors are under investigation. High genetic susceptibility, hormonal stimulation from breeding, and advanced age were responsible for the occurrence of spontaneous breast tumors in female mice deprived of the mammary tumor agent, according to a report by Dr. Walter E. Heston, Laboratory of Biology. In 1950, Dr. Heston reported development of a substrain (C3Hf) that had been deprived of the mammary tumor agent by foster-nursing newborn mice of the agent-carrying, high-tumor strain C3H on females of the agent-free, low-tumor strain C57Bl. Although it was anticipated that very few mammary tumors would occur in females of this substrain, 38 percent of the females of the first five generations that were intensively bred developed mammary tumors at an average age of 20 months. These observations showed that the mammary tumor agent, which possesses many attributes of a virus, is not essential for the appearance of all mammary tumors in mice.

Dr. Heston has now reported further studies to determine whether the agent is involved in the etiology of tumors developing in subsequent generations of substrain C3Hf and in three additional substrains.

Results of the 1950 report were substantiated by the observation that the incidence of mammary tumors in breeding females of the four substrains varied between 22 and 51 percent at approximately 20 months of age. In comparison, tumors have occurred in 97 percent of breeding females at 7.8 months of age, in strain C3H mice which have the mammary tumor agent.

There was a positive correlation between the number of litters produced by the females of the agent-free substrains and the incidence of mammary tumors. In substrain C3Hf, the incidence

ranged from 2 percent in virgin females to 44 percent in females that had borne 7 litters each.

Approximately 81 percent of the mammary tumors occurring in the agent-free females were types histologically characteristic of those in ordinary strain C3H females.

VETERANS STUDY CONFIRMS STATISTICAL RELATIONSHIP OF LUNG CANCER, SMOKING

The sharp rise observed in lung cancer in recent years has spurred intensive study of the causes of lung cancer. One approach to the problem involves epidemiological studies, which provide data on prevalence, incidence, distribution, and mortality of cancer. These data indicate possible relationships between cancer and certain environmental and other factors, such as age, sex, occupation, living habits, etc. In this way, information obtained by epidemiological studies contributes to the knowledge of the nature and extent of cancer in the population.

Confirmation of the findings of earlier studies demonstrating a statistical relationship between lung cancer and smoking was reported by Dr. Harold F. Dorn, National Institutes of Health.

Dr. Dorn is conducting an extensive and continuing statistical survey among 198,926 United States Government Life Insurance policyholders. In his first report, Dr. Dorn summarized data for 7,382 veterans who died between July 1954 and December 1956. These persons served in the armed forces between 1917 and 1940, and represented an age spread from 30 to 90 years. The majority were between 50 and 70. Of the group, 6,203 were smokers and 1,179 were nonsmokers.

Dr. Dorn's report included the following points: 1) The death rate from all causes of persons who used tobacco was 16 per thousand compared with 13.1 per thousand for persons who never smoked. Adjusted to take into account the differences in age distribution between the two groups, the death rate was 32 percent higher for smokers than for nonsmokers. 2) Persons who regularly smoked only cigarettes had the highest death rate of all groups of smokers -- 58 percent greater than the death rate for nonsmokers. 3) The lung cancer rate for regular smokers of cigarettes only was about 10 times the death rate for nonsmokers. 4) The death rates among regular cigarette smokers were closely related to the amount smoked. For example, death rates of persons who smoked more than 40 cigarettes a day were much higher than those of persons who smoked fewer than 10 cigarettes a day. 5) The death rate of persons who regularly smoked cigars and/or pipes was

not significantly higher than that of nonsmokers. Only the heaviest users of cigars and pipe tobacco had an appreciably higher death rate from all causes than nonsmokers. 6) Nearly two-thirds of the 6,203 deaths of tobacco users studied were attributed to diseases of the heart, blood vessels and kidneys. The death rate from coronary heart disease was found to be 63 percent greater for regular cigarette-only smokers than for nonsmokers. 7) Regular cigarette smokers who had stopped smoking cigarettes before the study began in 1954 had a lower mortality rate than those who continued to smoke. However, the rate was 30 percent greater than that of nonsmokers. 8) Regular cigarette smokers also had greater death rates from certain respiratory diseases such as bronchitis, pleurisy and emphysema, from ulcers of the stomach and duodenum, and from cirrhosis of the liver.

In addition to studying the use of tobacco, the continuing investigation will also explore possible statistical relationships between death rates and such environmental factors as occupations, work environments and characteristics of the communities in which the persons lived.

LUNG CANCER IN WOMEN ALSO RELATED TO SMOKING

A striking characteristic of lung cancer is its pronounced excess risk among males as compared with females. Lung cancer mortality in men is about 4 to 5 times that in women. Several studies have indicated that the risk of lung cancer mortality is greater among cigarette smokers than among nonsmokers. Previous reports in the liberature have suggested that sex differences in lung-cancer risk might be reconciled if the comparison were adjusted to take account of smoking history.

Mr. William Haenszel, Biometry Branch, has reported results of a retrospective study showing that there is a statistical association between cigarette smoking and lung cancer in women and that the lung cancer mortality rates for males and females are virtually the same when the sex comparison is restricted to nonsmokers.

In the study, medical and smoking histories of 158 women with diagnosed lung cancer in 29 institutions in the United States were reviewed. The two-year study, which began in March 1955, was substantially larger than previous investigations.

The largest and only statistically significant effect was associated with cigarette smoking history. None of the other items studied -- coffee drinking, occupation, migration, marital status, pregnancy history -- had relative risks approaching in magnitude

those associated with smoking history. These findings agree with 3 earlier studies of lung cancer in women.

AIRBORNE CARCINOGEN APPARENTLY TAKEN UP BY CELLS LINING LUNGS

Laboratory and statistical studies suggest that there is an agent in tobacco smoke tar associated with lung cancer in man. Other studies implicate air pollution in urban communities by a number of known carcinogens, including benzpyrene. To most investigators of this important public health problem, it seems likely that multiple factors are operative in the production of lung cancer.

The carcinogenic hydrocarbons, such as 3,4-benzpyrene, are carried on soots that are present as atmospheric contaminants in urban and industrial areas. These substances may, in part, account for the high incidence of lung cancer among city dwellers. Dr. Hans L. Falk, University of Southern California School of Medicine, has reported on a series of experiments to discover what becomes of 3,4-benzpyrene on soots when inhaled into human lungs.

Lungs heavily laden with soots were obtained at autopsy from 11 males, none of whom had succumbed to cancer of any site or to respiratory disease. Soots were removed from the lungs and analyzed for the presence of hydrocarbons, including 3,4-benzpyrene, a tumor-inducing compound in experimental animals. The results showed that 3,4-benzpyrene was absent.

Dr. Falk suggested that the disappearance of 3,4-benzpyrene from the soots in the lungs is due to a biological process whereby the compound is transferred from soots to the cells lining the lungs. This transfer may be accomplished by the action of an eluting agent or solvent that either enters the lungs on soots or is already present in the body, and that is capable of dissolving benzpyrene. This explanation is based on scientific evidence, which has shown that suitable solvents are present in contaminated air and that plasma protein dissolves 3,4-benzpyrene from soots. Whether the disappearance of benzpyrene from soots obtained from human lungs is a function of its carcinogenic property remains to be determined.

TOBACCO TAR ENHANCES TUMOR-PRODUCING POTENCY OF BENZPYRENE IN MICE

Dr. Alfred Gellhorn, Columbia University College of Physicians and Surgeons, has reported a two-year study supporting the concept that air pollution, together with cigarette smoking, accounts for the higher incidence of lung cancer among city dwellers. In a series

of experiments, various combinations of tobacco tar obtained by the combustion of cigarette tobacco at 500 to 700° F. and the known carcinogens, benzpyrene and croton oil, were applied to the backs of mice. The animals were observed for the development of skin tumors, such as papillomas and carcinomas.

Under the conditions of this study, tobacco tar alone failed to demonstrate marked carcinogenic activity. Benzpyrene plus tobacco tar and also benzpyrene plus croton oil produced a significantly greater proportion of papillomas and carcinomas than did benzpyrene alone. The rate of development of carcinomas in mice bearing papillomas was significantly greater among those receiving benzpyrene plus tobacco tar than those receiving benzpyrene alone or benzpyrene plus croton oil.

Dr. Gellhorn concludes from these observations that tobacco tar has cocarcinogenic activity in the production of skin tumors in mice given a carcinogenic dose of benzpyrene.

HAMSTERS RECEIVING DMBA IN LUNGS DID NOT DEVELOP LUNG CANCER

Dr. Giuseppe Della Porta, Chicago Medical School, has reported on laboratory studies designed to administer suspected carcinogens directly into the lungs of animals of a species that does not ordinarily develop spontaneous lung cancer or pulmonary infections. The results showed that instillation of tobacco tar into the tracheobronchial tree of hamsters failed to produce lung cancers, and that the carcinogenic hydrocarbon, 9,10-dimethyl-1,2-benzanthracene (DMBA) produced a variety of pathological changes including tumors.

In the technique that was developed in this study, DMBA and a cigarette tobacco tar condensate, made up in gelatin colloid suspensions, were administered repeatedly alone or in combination into a tube leading directly to the tracheobronchial tree of male and female Syrian golden hamsters. This procedure simulates the manner in which carcinogens might normally reach the lungs. The animals were left to die spontaneously or were sacrificed when moribund. At autopsy the entire respiratory tract, including the hypopharynx and the upper portion of the esophagus, was removed and examined microscopically.

The results showed that instillation of DMBA produced a variety of pathological changes in the tracheobronchial epithelium, ranging from an abnormal multiplication of normal cells to frank cancer of the trachea and bronchi. The dosage of the compound appeared to be an important factor in the induction of cancer. Forty-five repeated doses of 50 micrograms of DMBA yielded only borderline carcinogenic action on the tracheobronchial epithelium; 17 doses of 100 µg.

induced a significant number of tumors in this location.

Administration of tobacco tar produced no histologically demonstrable lesions.

CHROMIUM VI SUGGESTED AS CAUSATIVE AGENT IN LUNG CANCER

Laboratory studies to determine the agent responsible for the production of lung cancer among workers in the chromate-producing industry have suggested chromium as the carcinogenic agent. About a year ago, scientists of the Environmental Cancer Section reported that dusts of crude chromite ore dispersed in physiologic buffer solutions became soluble when aerated. Deposits of biologically inert compounds of chromium might accumulate in the lungs when inhaled as dusts. Action of body chemicals on these compounds might release a more active form of chromium (chromium VI) in small amounts over long periods.

Dr. W.C. Hueper has summarized the results of experiments in which implants of chromite roast (product of the process of roasting chromite ore) were made in rats either in the pleural cavity or muscle tissue of the right thigh. All animals surviving an observation period of two years were killed and autopsied.

Among the group of 25 male rats receiving intrapleural implants, four survived into the cancer-bearing period and two showed squamous-cell carcinomas coexisting with sarcomas of the lung. Of 31 female rats receiving intramuscular implants in a thigh, 29 were alive at the first appearance of tumors, and three fibrosarcomas at the site of the injection were observed. These observations suggest that chromite ore roast contains chromium in a form that exerts a sufficiently strong and prolonged effect upon exposed tissues to cause cancers.

More recent experiments have produced sarcomas in 6 of 35 rats that received implants of synthetically prepared sintered calcium chromate. The sarcomas developed within five to six months around these chromate deposits. Observation of the surviving rats will continue for two years.

INHALED NICKEL DUST CAUSES RESPIRATORY TUMORS IN LABORATORY ANIMALS

Workers employed in nickel smelting plants sometimes develop tumors of the nasal cavity, the paranasal sinuses, and the lungs following prolonged inhalation of nickel dust, fumes and vapors. Tumors of the bone, connective tissue, nerve tissue, and muscle have been produced

experimentally in animals after parenteral administration of metallic nickel. However, in such experiments, the tumors were not produced through a route of administration encountered under occupational conditions of exposure in man and were not the same types observed in man. Dr. W. C. Hueper has shown that inhalation of nickel dust by animals produces respiratory tumors similar to neoplastic reactions seen in nickel smelter workers.

Guinea pigs, rats, and mice were exposed to an atmosphere containing a controlled amount of powdered metallic nickel for 6 hours per day, 4 to 5 days a week until death. The maximum period of exposure was 21 months. Autopsies were performed on all animals, and internal organs and tissues showing gross pathological changes were examined histologically.

Virtually all guinea pigs and many rats subjected to an atmosphere of nickel dust developed multicentric pulmonary adenomatoid and hyperplastic lesions. One guinea pig developed a frank pulmonary carcinoma and another with extensive adenomatoid changes in the lung had the same type of tissue in an abdominal lymph node. These lesions are attributable to nickel dust inhalation and can be considered to be etiologic and organic equivalents of respiratory neoplastic reactions seen in nickel smelter workers.

SHALE OIL CONTAINS CARCINOGENS OTHER THAN BENZPYRENE

Laboratory studies have shown that crude American shale oil and some of its distillation fractions are carcinogenic for experimental animals. Scientists of the Environmental Cancer Section have conducted an investigation to determine whether shale oil fractions free of the known carcinogen benzo(a)pyrene contain carcinogenic properties. Dr. W. C. Hueper has reported a study in which a total of 16 fractions of shale oil were injected intramuscularly into groups of mice. The animals were then observed for the development of malignant tumors and other pathological changes. Several shale oil fractions that were free of benzo(a)pyrene induced sarcomas in mice. Dr. Hueper concludes, therefore, that the carcinogenic power of shale oil depends only in part on its benzo(a)pyrene content and that other, as yet unidentified, constituents contribute to the carcinogenic effect of crude shale oil.

CIGARETTE SMOKE TAR DOES NOT PRODUCE CANCER IN HAMSTER ORAL POUCH

Scientists of the University of Louisville School of Medicine observed that tobacco maintains the most consistent association with mouth cancer, as compared with alcohol and diet. Since cigarettes are the most widely used form of tobacco in this country, a study of the effects of cigarette smoke tar in the oral pouch of the golden hamster was undertaken. Cotton wads impregnated with cigarette smoke tar were sewn into the right oral pouch of one group of hamsters. In another group, the pouches were painted with cigarette tar. Dr. Condict Moore reported that a large majority of the hamsters survived a year or more and that no tumors developed in either group. It was concluded that, by the method used, whole cigarette smoke tar alone does not appear to cause cancer in the hamster cheek pouch.

STUDY SHOWS DOWNWARD TREND IN LEUKEMIA RATES

Dr. Alexander G. Gilliam, Field Investigations and Demonstrations Branch, reported results of an epidemiological study showing a downward trend in leukemia death rates in the United States.

Analysis of the geographic distribution of leukemia deaths showed significantly low rates in the Southeastern portion of the country and significantly high rates in the North Central and Northeastern states. This geographic pattern was not altered when the rates for the metropolitan and nonmetropolitan counties were considered separately.

With regard to trends, Dr. Gilliam reported that there was a continued increase between 1921 and 1955 in mortality attributed to leukemia. This increase was greater among the nonwhite, whose rates now equal those recorded 10 to 15 years earlier for the white. Since about 1940, the rate of increase has regularly declined among the white of all ages. This is partly due to actual decline in death rates for infants since 1940 and for children 1-4 years of age since 1950, and partly to a decrease in the rate of increase for all other age groups except for those of 75 years or older. Among the nonwhite, the decline in rate of increase began about 1948 and is only clearly evident for males under 5 and females under 35.

Dr. Gilliam suggested that if these trends persist, they will eventually result in stabilization or actual decline in leukemia mortality for all ages. The trends appear to provide no support for any theory that postulates a recent sharp increase in

leukemogenic factors affecting the environment of Americans in general. On the contrary, he believes that the observed trends are consistent with a hypothesis of recent stabilization or actual decrease in general population exposure to whatever causes operate to induce the disease.

STUDY CONFIRMS REPORTED DECLINE OF STOMACH CANCER RATES IN UNITED STATES

A comprehensive epidemiological review by Mr. William Haenszel, Biometry Branch, has confirmed the reported decline of stomach cancer in the United States. The review is concerned chiefly with results obtained in American studies, but comparison is made with data from some 23 foreign countries, including England, Wales, Japan, Iceland, the Scandinavian countries, and others. Stomach cancer rates in relation to such factors as age, sex, race, geographic region, nation of origin, heredity, socio-economic status, etc. are reviewed.

Following are some of the key findings presented in the paper: stomach cancer rates in the United States have steadily declined for both men and women during the past several decades, a trend duplicated in some but not all foreign countries (age-adjusted rates per 100,000 population for the U. S. were 28.8 in 1930, 26.5 in 1935, 22.5 in 1940, 19.9 in 1945, 16.0 in 1950, and 13.0 in 1955); stomach cancer occurs more frequently among persons in the lower socio-economic groups but does not seem to be associated with occupation; there is no significant variation in the stomach cancer rates for urban and rural residents; the stomach cancer rates are higher in the northern section of the United States, and this trend parallels the international pattern of the geographic distribution of gastric cancer.

On the basis of these results, Mr. Haenszel suggested two possible avenues for further study of the epidemiology of stomach cancer; prospective studies of the role of endogenous factors (heredity), and retrospective studies of the influence of diet. Mr. Haenszel indicated that none of the available data rule out the possibility that some item(s) in the diet may be associated with the production of gastric cancer. Retrospective studies of the dietary histories of stomach cancer patients could resolve some apparent inconsistencies in the information now available and lead to the recognition of some food item or combination that would fit into the total epidemiological pattern for stomach cancer.

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LUNG CANCER RATE
HIGHER IN URBAN
DENMARK THAN U.S.

Lung cancer, the leading cause of death from cancer among men in the United States, occurs at a slightly higher rate among Copenhagen men than among men in metropolitan areas of the United States. Breast cancer, the leading cause of cancer deaths among United States women, occurs at a lower rate among Danish women than among United States white women.

These findings were reported by Danish scientists, Dr. Johannes Clemmesen and Dr. Arne Nielsen, of the Finsen Institute and Radium Hospital, Copenhagen, who studied cancer incidence in Denmark and adjusted the data to the United States population of 1950, permitting a direct comparison between the incidence of cancer in Denmark and the United States.

Analysis of the data comparing cancer incidence in Copenhagen with cancer incidence in 10 United States metropolitan areas as reported by Dr. Harold Dorn, NIH, and Sidney Cutler, Biometry Branch, National Cancer Institute, in 1955 showed, in part: 1) Rates for cancer of the buccal cavity and pharnyx are lower for men and women of all races in Copenhagen than in the United States. 2) White men and women in the United States show a lower incidence of gastric cancer than the Copenhagen population, while for nonwhites it is approximately equal. Women of all races in both countries show lower gastric cancer rates than men. 3) Copenhagen men show a slightly higher lung cancer incidence than do men in United States metropolitan areas. For women, the difference is negligible. 4) Breast cancer occurs among Danish women at about the same rate as it does among nonwhite women in the United States -- that is, lower than the rate for U. S. white women. 5) In both the United States and Copenhagen, nonwhite women appear to show a higher incidence of uterine cancer than do white women, although the relatively high proportion of unspecified cases reported in the United States makes analysis difficult. 6) Prostatic cancer is diagnosed more frequently in the United States than in Copenhagen.

The total incidence rate for all malignancies reported in Denmark was highest in Copenhagen and lowest in rural areas.

CARCINOGEN UTILIZED
DIFFERENTLY IN
GUINEA PIGS, MICE

Other studies on the causation of cancer are designed to produce information on the process by which carcinogens cause cancer. N-2-fluorenylacetamide is a potent carcinogenic agent which produces many different types of tumor in the rat but fails to cause cancer

in guinea pigs. Dr. John Weisburger, Laboratory of Biochemistry, has studied the differences in the metabolism of N-2-fluorenylacetamide by these species.

The rat changes N-2-fluorenylacetamide into four major compounds, while the guinea pig excretes only one of these four metabolites to any significant degree. The principal identified end products of both species are made by hydroxylation of the parent compound, and those excreted by the rat and not by the guinea pig are known as orthohydroxylated amines.

Earlier reports by British scientists suggest that orthohydroxylated amines of beta-naphthylamine, an extremely powerful carcinogen for the human bladder, are in fact the true cancer-producing agents. The findings of the National Cancer Institute biochemists suggest that production of this type of compound may be responsible for cancer production in the rat, and the lack of such change in the guinea pig could be the reason why this species does not get cancer when fed N-2-fluorenylacetamide. Solution of the problem, however, will require biological testing of the hydroxylated compounds in both rats and guinea pigs. The organic chemist has not learned how to synthesize these compounds, so the investigators use rats as a factory for producing them but still find it difficult to separate the specific compounds from one another. This work is now in progress.

CHARACTERISTICS OF CANCER

INFECTION SHOULD BE SUSPECTED IN FEVER OF ACUTE LEUKEMIA

Studies of the metabolism of cancer patients and clinical investigations of manifestations of various types of cancer provide practical information about the behavior of a cancer and its effect upon the patient, and fundamental information about the nature and development of cancers.

A year-long study has revealed that infection is a major cause of fever in acute leukemia patients. In a collaborative project with the National Institute of Allergy and Infectious Diseases, scientists of the General Medicine Branch have studied the occurrence of fever, infection, and antibody response in acute leukemia.

A group of 36 children and adults under treatment for acute leukemia in the Clinical Center between March 15, 1955 and March 1, 1956, constituted the patient material for this investigation. Dr. Richard T. Silver reported that on the basis of various clinical and laboratory tests, the 92 febrile episodes observed in these patients were divided into three categories: 1) proved infection; 2) presumptive infection; and 3) fever of undetermined origin.

Two patients had no febrile episodes during the period covered by the study. The remaining 34 patients showed temperature elevations approximately 45 percent of the time. Nearly two-thirds of the febrile episodes (59) appeared to be related to infection; while in the remaining 33 episodes, bacterial, fungal, or viral infection could not be demonstrated. Age and type of leukemia did not influence the frequency of febrile episodes. However, more febrile episodes related to proved or presumptive infection developed in adults than in children.

The frequent occurrence of bacterial infection during the course of acute leukemia led the investigators to study cellular and humoral defense mechanisms in the patients. Although the antibody response of leukemia patients as a group was found to be less than that of a group of normal controls, some patients exhibited excellent antibody response when challenged by specific antigens. In a given patient, there was no correlation between antibody response and the frequency of bacterial infection.

At the conclusion of the study, 14 of the patients were dead, 10 as a result of overwhelming infection. In this group of patients, infection ranked with hemorrhage as a leading cause of death.

These findings led the investigators to the following conclusions: 1) In the initial evaluation of a febrile acute leukemia patient, a negative physical examination does not exclude infection as a cause for fever. 2) Significant episodes of fever in the patient with acute leukemia should be considered the result of infection until proved otherwise. In the absence of signs and symptoms, blood cultures and chest roentgenograms are the most valuable laboratory aids in the detection of infection. 3) The use of long-term prophylactic antibiotic therapy to combat fever and infection may not be advisable in patients with acute leukemia. Such therapy may produce alterations in normal flora and result in superinfections, both of which are to be particularly feared in acute leukemia. 4) Appropriate management for persistent fever of undetermined origin requires continual reappraisal to insure that a superimposed infection, masked by preceding unexplained fever, has not gone undetected.

TOXICITY OF AMINOPTERIN RESULTS FROM IMPAIRED FOLIC ACID METABOLISM

Various studies of the folic acid vitamins reported in the literature have shown that 1) folic acid is essential to growth, 2) folic acid is converted to folinic acid (citrovorum factor) in the body, 3) the folic acid antagonist, aminopterin, which has temporary usefulness in the management of acute leukemia of childhood, blocks the conversion of folic to folinic acid, and 4) administration of aminopterin results in increased urinary excretion of a test dose of folic acid.

Dr. Paul T. Condit, General Medicine Branch, has reported results of a systematic study of the metabolism of folic acid in man and the effect of aminopterin on this metabolism.

In the study, folic acid was given intravenously and, subsequently, aminopterin was given orally to normal subjects and patients with leukemia and other neoplastic diseases, all of whom were ambulatory, in good nutritional status, and without evidence of renal disease. Venous blood and urine samples were collected for analysis.

The intravenous administration of folic acid was followed by a rise in plasma folic acid concentration and an increase in the urinary excretion of folic and folinic acids. Two thirds of the injected compound was excreted at a rate that was proportional to the plasma concentration.

The administration of aminopterin resulted in an increase in the urinary excretion of injected folic acid. The increase in urinary excretion of folinic acid that follows the injection of folic acid was inhibited.

The only difference observed between leukemic and nonleukemic subjects in the metabolism of injected folic acid was a greater increase in the urinary excretion of folinic acid in two patients with chronic myelocytic leukemia.

Other investigators have reported that injection of either folic or folinic acid before aminopterin is given exerts a protective action against the toxicity of aminopterin, but only folinic acid is effective after aminopterin administration. Dr. Condit suggests that the toxic effects of aminopterin in animals and man appear to be due to inhibition of the conversion of folic to folinic acid. He suggests further that the therapeutic effects of aminopterin are due to the same mechanism.

SERUM B-12 CONCENTRATION HIGHEST IN PATIENTS WITH ONE TYPE OF LEUKEMIA

In a survey of a large group of patients with malignant diseases, serum vitamin B-12 concentrations have been found to be much higher in patients with one type of leukemia than in those with other forms of cancer or in well persons.

Dr. I. Bernard Weinstein and Dr. Donald M. Watkin, General Medicine Branch, have reported that this result was obtained when the amount of vitamin B-12 was measured in the blood serum of 205 persons, 174 of whom had malignant diseases. In 11 out of 12 patients with chronic myelocytic leukemia, B-12 concentrations were markedly elevated, ranging from 2 to 40 times higher than normal.

In two patients with this disease, vitamin B-12 concentrations were measured periodically during the course of therapy. A clinical remission in one patient resulted in a reduction in B-12 concentration to the normal range.

Patients with some other types of cancer also showed elevated B-12 concentrations, but in no case were they so consistently high as in patients with chronic myelocytic leukemia. Elevated B-12 concentrations in patients with other forms of cancer usually occurred when the disease had metastasized (spread) to the liver.

The reason for the high B-12 concentrations observed in these leukemia patients is not clearly understood.

ROLE OF SERUM STUDIED IN RESISTANCE TO INFECTION

Scientists of the Laboratory of Chemical Pharmacology have studied an unidentified factor in human serum which nullifies the characteristic effects of endotoxic polysaccharides (bacterial poisons), when serum

and polysaccharides are incubated together in the test tube. This factor may be important in resistance to cancer.

Dr. Maurice Landy reported that this factor is not properdin (a substance in blood serum important to resistance to infection), since serum completely lacking in properdin is equally effective in inactivating endotoxic polysaccharides.

Further study showed that the factor is susceptible to heat; heated serum did not counteract the effects of endotoxic polysaccharides.

The significance of this study to cancer research lies in the fact that endotoxins produce the characteristic host reactions of certain infections and may also affect the host by increasing resistance to infection. These endotoxins, which are complex carbohydrates, also have the capacity to damage some cancer cells. Extensive research is being conducted to find out how the host attempts to counteract the effect of endotoxic polysaccharides, because the results of this research may help to explain the mechanism of resistance to infection, and possibly may be involved in resistance to cancer.

STUDY MOUSE PLASMA CELL NEOPLASMS RESEMBLING HUMAN MULTIPLE MYELOMA

Laboratory studies also yield information about the characteristics of cancerous and normal growth processes. One of the striking features of human multiple myeloma is the frequent occurrence of large amounts of an abnormal plasma protein. Although this aspect of the disease has been the subject of extensive research, many fundamental questions about the plasma protein abnormalities remain unanswered.

A group of scientists in the General Medicine Branch have been studying several mouse tumors that closely resemble human multiple myeloma. These are of particular interest because they provide an experimental approach to the problem of the human form of the disease. Dr. Daniel Nathans has now reported the results of studies with one of these mouse neoplasms to ascertain whether the myeloma protein is produced by the tumor cells or is a response of other host tissues to the effect of the tumor.

In the study, an isotopically labeled amino acid was administered to mice bearing a plasma cell tumor. The animals were sacrificed at intervals from 10 minutes to 16 hours. Myeloma protein was isolated from the plasma and tumor tissue of each animal, and the specific activity of the labeled amino acid in each sample was measured.

Analysis by paper chromatography, electrophoresis, and ultracentrifugation revealed that the myeloma proteins obtained from plasma and from tumor tissue were homogeneous and had identical properties. Measurement of the specific activities of the plasma myeloma protein and the tumor myeloma protein indicated that the protein was synthesized in the tumor.

STUDY MECHANISM OF AMPHENONE ACTION ON RABBIT UTERUS

Amphenone, a compound developed by scientists of the Endocrinology Branch, suppresses the hormone production of the adrenal glands and is a prototype of drugs that may be developed as effective substitutes for surgical removal of the adrenals.

Dr. William W. Tullner conducted a study to determine whether amphenone directly affects the rabbit uterus to produce progestational change or whether its action is mediated indirectly by secretion of progestogens from another endocrine organ. He found that the action appears to be due to excess secretion of progesterone by the adrenal glands.

In the study, the effect of amphenone was observed in rabbits that had been adrenalectomized and ovariectomized. Dr. Tullner reported that when the adrenals were removed, administration of amphenone failed to induce a progestational effect. When adrenals were not removed and cortisone was administered in conjunction with amphenone, amphenone failed to induce progestation. This observation suggested that cortisone caused a decreased rate of secretion of ACTH, thereby inhibiting the production of adrenal steroids, including the progestogens. However, administration of ACTH also failed to induce progestational activity in the rabbit endometrium. Removal of the ovaries had no effect on uterine response to amphenone.

Dr. Tullner suggested that amphenone exerts its effect by blocking the biosynthetic pathway between progesterone and corticosterone.

CHARACTERISTICS OF HIGH AND LOW TUMOR-PRODUCING CELL LINES ARE STUDIED

Tissue culture is a valuable tool for studying the development of malignancy because it permits the study of cells grown outside the body. Tissue culture facilitates study of the development of malignancy, effectiveness of anticancer drugs, and differences between normal and cancer cells.

Scientists of the Laboratory of Biology have previously reported studies of two cell lines grown in tissue culture from a single mouse cell and possessing marked differences in ability to produce tumors. The "high" line grew into malignant tumors in 97 percent of the mice in which it was injected; the "low" line grew as tumors in only 1 percent of the mice injected.

Dr. Katherine K. Sanford has summarized the present state of knowledge of the neoplastic transformation of normal cells in vitro, including results of studies of her laboratory and those of others. She reported that the difference in ability of the two cell lines to grow as tumors when injected into mice appears to be related to different metabolic patterns and to hereditary changes in morphology, chromosomal composition, and enzyme activity.

In collaboration with Dr. Ruth Merwin, of the same Laboratory, Dr. Sanford found that the two cell lines differed in growth characteristics; the cells of the "high" line proliferated more rapidly during the first few days after implantation into mice, spread more rapidly into the surrounding tissue, and induced enlargement and convolution of nearby blood vessels, which did not occur with "low" line cells.

These observations led to the conclusion that cells of the high line differ from those of the low by producing a tumor before immunity develops in the host. It is well known that an established tumor can continue to grow after immunity develops.

Although cells of the two lines grew at different rates in mice, the rate of growth of both cell lines was about the same in the test tube. Hence, the difference in tumor-producing ability between the two lines may be due to a difference in their response to some growth-controlling factor in the host mouse.

Investigation of the metabolic properties by Dr. Mark Woods and Dr. Dean Burk, of the Laboratory of Biochemistry, showed that the "high" line cells utilized approximately three times as much glucose as "low" line cells. There appears to be some correlation between the high glycolytic activity of the "high" line and the rapid growth of these cells into palpable tumors, and the lower glycolytic activity of the "low" line cells and their increased tumor latent periods.

Dr. B. B. Westfall and his associates of the Laboratory of Biology reported that enzyme activity was low in both the "high" and "low" strains. However, a significant difference was noted in derivatives of the "high" strain. After three years of growth in vitro, one of the "high" line substrains showed an arginase activity 20

times greater than that of the parent strain; another substrain showed a 400-fold greater arginase activity.

Other differences were noted by Dr. Sanford in morphology and chromosome numbers and types.

The findings suggest that the two lines from one cell have undergone diverse neoplastic transformations in vitro, that the cells of the "low" line as compared with the "high" may be less altered from the normal in their responsiveness to growth restraints in vivo, and that this characteristic may be associated with their different metabolic patterns, Dr. Sanford concluded.

GROWTH CHARACTERISTICS SPECIFIC FOR EACH MAMMALIAN CELL STRAIN

The usefulness of tissue culture has been increased by technological advances that have made possible the growth and maintenance of massive long-term cultures under controlled conditions, and the establishment of pure strains of cells each grown from a single cell.

Jay C. Bryant, Laboratory of Biology, has reported studies of the growth of mammalian cells in free floating, fluid-suspension cultures. The first study was carried out on 5 types of established cell strains or clones, including three derived from mouse cells and two derived from human cells. The second study was done on the same 5 strains and two additional strains derived from mouse cells.

The cells are grown freely suspended in a continuously aerated and agitated nutrient fluid. A shaker originally devised for pilot-plant use in the antibiotics industry was adapted for this purpose, and the principles involved in the growth of cells in this system are similar to those established by the antibiotics industry for growth of micro-organisms. It is thus possible that as culture media are developed, a number of cell strains may be grown on an extremely large scale.

The various cell strains showed major differences in growth characteristics, which appeared to be specific for each cell strain used in the studies. In the manner of growth, the cultures ranged from single-cell suspensions to rather large, compact aggregates. Only single-cell suspensions were well suited for quantitative studies.

The formation of noncellular protein debris was apparently a characteristic of fluid-suspension cultures of human skin

epithelium regardless of culture condition, but seldom formed in cultures of mouse fibroblast or mouse liver cells. The debris appeared to originate from the serum used in the culture medium.

The specific animal cell strains studied had characteristic growth rates. Smooth growth curves, reflecting uniform rates of increase, generally characterized mouse fibroblast and mouse liver cell clones, while irregular growth curves were typical of skin strain cultures.

Mr. Bryant concluded from the results on growth rates that the data are consistent with the concept of a growth cycle that involves a sequence of phases, such as has long been recognized as useful in characterizing the growth of micro-organisms, including an initial lag phase, a logarithmic or rapid growth phase, a stationary phase, and a phase of decline.

Average rates of glucose utilization also appeared to be specific characteristics of established cell strains. Cultures of cells of human skin epithelium consistently utilized glucose at a high rate, mouse liver cells at an intermediate rate, and mouse fibroblast cells at a low rate. Such consistent differences in rates of glucose utilization suggest concomitant differences in metabolism.

GROWTH BEHAVIOR OF ANIMAL TUMOR CELLS AIDS METASTASIS STUDY

An approach to the problem of the metastasis of cancer is the study of the invasive capacity of tumor cells of experimental animals. Various transplantable lymphocytic neoplasms in mice exhibit differences in capacity to invade the tissues of the host and in growth characteristics.

Dr. Emma Shelton, Laboratory of Biology, has reported studies on the behavior of several mouse lymphocytic tumors in an effort to define more precisely some of the differences among them. The studies were conducted with a localized lymphocytic neoplasm (lymphosarcoma) and two generalized lymphocytic neoplasms (lymphocytic leukemia).

A study was made of the invasive capacity of leukemic cells in an artificial system, the diffusion chamber, where a cellulose-membrane filter is used as a synthetic, porous barrier on which cells are grown. The two more invasive lymphocytic neoplasms, Ll210 and L#2, penetrated the cellulose membrane more quickly than

did the cells of the less invasive lymphocytic tumor, L#1, which remained localized. No correlation could be established between cell size and ability to penetrate the filters, and the mode of progression of the cells through the filters was the same for all three tumors.

The behavior of the less invasive tumor, L#1, was distinctly different from that of the other two. Notable features of the growth of L#1 cells were an increased viscosity of the fluid in the chamber, the formation of fibers, and the growth of tumor cells within the normal host cells.

Dr. Shelton reported further studies of the growth characteristics of the three tumors. Cell-population growth was studied by growing the cells in the peritoneal cavities of mice. The mice were injected with a dye at predetermined intervals, were killed, and the fluid was removed for analysis. The growth of the two generalized lymphocytic neoplasms closely paralleled each other, in that the cells immediately started to grow rapidly and doubled their population in 12 to 14 hours. In contrast, the cells of the localized neoplasm did not start to multiply for about 66 hours, after which they reproduced rapidly, but did not achieve the level of growth of the other two tumors.

Dr. Shelton suggested a relationship between the lag period in the growth of the L#1 tumor and the inability of the cells of this tumor to become established at different sites in the host.

TSPA REDUCED LUNG METASTASES IN TUMOR-BEARING MICE

Scientists of the Surgery Branch have investigated the possible association between surgical removal of a malignant neoplasm and an increase in the dissemination and growth of metastatic lesions.

Dr. William M. Kramer reported results of a study in which triethylenethiophosphoramide (TSPA) was moderately effective in reducing the number of lung metastases in mice bearing transplanted tumors. TSPA was used because of its effect in diminishing the size of metastatic lesions and improving the clinical course of the disease, especially in patients with breast carcinoma.

In the study, mice were inoculated in the tail vein with S-91 melanoma tumor cells. TSPA was given intraperitoneally in varying dosage schedules. Animals were sacrificed a month after inoculation of the tumor and the lungs, the only organs that contained metastases, were excised and examined.

The results showed that doses from 1 to 20 micrograms per gram of body weight were increasingly effective in reducing the number of metastases. With all doses tested, effectiveness increased to a maximum on the second day and then diminished gradually over a period of 2 weeks. Under optimal conditions, some animals which without treatment might have been expected to have 100 or more tumors had none.

Tests with several other tumors in mice, rats, and rabbits showed that the susceptibilities to TSPA differed. Hence, Dr. Kramer concluded that considerable caution must be employed in extrapolating the results of animal experiments with TSPA to the human situation, and he emphasized the need for more information about the susceptibilities of human tumors to such therapy.

TSPA ALSO REDUCED METASTASIS FOLLOWING PRIMARY TUMOR REMOVAL

In another group of laboratory experiments, Dr. Kramer and Dr. William E. Schatten, Surgery Branch, showed that removal of a primary tumor stimulates the development and growth of metastatic lesions. The studies were done on mice about three weeks after they were injected with tumor cells intramuscularly in a hind leg. Subsequently, the mice were sacrificed and their lungs examined for metastases.

The first study showed that in mice on which amputation of a normal leg or on which no amputation was performed, the frequency and number of metastases were not significantly different. However, removal of the leg bearing the transplanted tumor resulted in a significantly increased frequency, number, and size of pulmonary metastases. Removal of the primary tumor at the end of three weeks resulted in larger metastases than those observed in mice in which the primary tumor was present for six weeks. It was concluded that a primary tumor exerts an inhibitory effect on distant metastases and that removal of the tumor acts as a stimulus on the metastatic process.

The second study showed that no effect on the number of pulmonary metastases resulted from anesthesia, cortisone administration, or operative procedure.

The third study showed that the anticancer drug, triethylenethiophosphoramide, (TSPA), was effective in reducing the number of pulmonary metastases when administered several hours after removal of the primary leg tumor. Metastases were completely prevented in some mice. The scientists suggest that the conditions of this

experimental system, in which the effectiveness of a chemotherapeutic agent was ascertained, simulate clinical postoperative situations.

DNA HIGHER IN MALIGNANT THAN IN NORMAL TISSUES

Biochemical studies include research on the chemical components of cells. Studies of the complex molecules of proteins and nucleic acids, which are responsible in large measure for cell growth, function and reproduction, produce additional information about the characteristics of cancer.

Dr. Cecilie Leuchtenberger, Western Reserve University, reported on the investigation of chemical components of normal and malignant cells by a special technique called microspectrophotometry, which combines the microscope with a spectrometer. The analyses were done directly on microscopic sections of fixed intact single cells or parts of cells so that, without destroying either the architecture of the cells or their relationships within a tissue, a comparison of cell structure with its chemical composition could be made.

The study showed that the amount of desoxyribonucleic acid (DNA), an essential constituent of the heredity-bearing units called chromosomes, was remarkably constant in normal cells of all tissues, irrespective of their physiologic function. In contrast, the DNA content of precancerous lesions and malignant tissues was found to be considerably higher.

Through the techniques used, it was possible to show differences between a precancerous lesion and a malignant lesion (senile keratosis of the skin). Values were also obtained on the DNA content in a stomach cancer, a metastasis from this tumor to a lymph node, and the surrounding normal stomach tissue. The tumors showed the presence of larger amounts of DNA.

Dr. Leuchtenberger concluded that DNA values are related to the cell multiplication processes and hence values higher than normal cannot be considered a specific criterion for diagnosis of cancer. However, an increase in DNA values in cells of an adult tissue should evoke suspicion that tumorous growth exists, unless regeneration is to be expected. Studies of this kind may be helpful in diagnosis of borderline cases of cancer.

RAT EXPERIMENTS DEMONSTRATE VALUE OF SYNTHETIC DIETS

According to a report by Dr. Jesse P. Greenstein and his colleagues of the Laboratory of Biochemistry, studies with rats have shown the nutritional role of several components in synthetic diets. Some of the findings reported are as follows: 1) Rats were fed chemically defined diets containing roughly 20 percent amino acids as the sole source of nitrogen. They bred, lactated, and reared young. Three successive generations were reared on the optimal diet. 2) The ratio of nitrogen retained to the average daily weight gain was constant for animals on three diets which differed in amino acid composition, even though each diet produced different rates of growth. 3) No amino acid studied was "nonessential" for maximal growth. 4) Growth responses were similar when the diet contained sucrose, glucose, fructose, or invert sugar. Increasing quantities of D-glucosamine in the diet were associated with a progressively decreased dietary intake by the animal. 5) The amino acids L- and D-arginine both appeared to render the diet nutritionally more adequate.

With regard to human use, the water-soluble diets are found to be edible and should prove of value in the care of patients with alimentary problems. They are particularly significant in that they open possibilities for a completely synthetic diet to be administered parenterally to patients who cannot eat. In their present form, the diets offer many possible advantages by virtue of their small bulk, such as economy of storage for civilian defense, arctic habitations, and space travel. Animals on the diets pass very small amounts of excreta, indicating a further advantage for human use under special conditions.

IRON LOST FROM BLOOD RELATED TO IRON IN TUMOR AND TO ANEMIA

Many patients with cancer have anemia at some stage of their disease. In some instances the anemia results from hemorrhage, but more often the cause is unknown. In recent years, it has become increasingly apparent that in leukemic patients erythrocyte (red blood cell) destruction plays a dominant role in the development of anemia.

Scientists of the Laboratory of Biochemistry have been investigating the nature of the process of red blood cell destruction. Dr. Robert E. Greenfield has reported studies in which radioactive iron (Fe^{59}) labeled red cells were injected intravenously into tumor-bearing animals. The data showed that some of the rat tumors had a large

amount of iron which was derived from red cells. The extent of this process varied greatly from tumor to tumor. When the process was extensive it was accompanied by anemia and the animal died while the tumor was still relatively small. When the process was slower, the tumors became larger, the onset of anemia occurred later, and the animals lived a longer period of time.

It was also found that a high proportion of the Fe^{59} which had left the blood stream could be found in the area of the tumor. The conclusion drawn from this study was that the loss of Fe^{59} labeled red cells from the blood stream was correlated with the amount of radioactive iron in the tumor, the onset of anemia, and the development of malnutrition in the host animals. Further studies into the mechanism of the process are in progress.

FETAL HOMOLOGOUS TISSUE PROTECTS MICE AGAINST FATAL RADIATION DOSE

Studies of the effect of radiation on living tissue are particularly important in cancer research. Such information contributes to improved therapy and diagnosis as a result of advances in techniques of administering radiation and new knowledge in host response to radiation.

Several investigators have reported that bone marrow injected into lethally irradiated mice can prevent or forestall death. Inoculation of isologous marrow, marrow from a member of the same inbred strain, produces a lasting recovery in irradiated mice, although their life span is shortened. Homologous marrow, marrow from a different strain of the same species, affords only a temporary recovery. Most animals that receive homologous marrow succumb during a secondary phase of the irradiation syndrome. The question whether the secondary phase is the result of an immune response of the host to the tissue of the foreign strain or of the graft against the irradiated host is being investigated in several laboratories.

Miss Delta E. Uphoff, Laboratory of Biology, has reported that fetal homologous hematopoietic tissue protects mice against lethal x-ray dosage. The study was based on the assumption that, if the graft-versus-host reaction exists when adult tissue is used, fetal hematopoietic tissue might preclude the secondary phase reaction. This would occur because fetal tissue would be immunologically immature and thus unable to evoke a reaction in the host.

In Miss Uphoff's studies, hybrid mice, 3 to 4 months old, were exposed to 800 roentgens of x-irradiation, a dose lethal to 98 percent of mice of this strain. Within 3 hours after irradiation, the

test mice received inoculations of hematopoietic tissue. Irradiated mice that received no inoculation served as controls. The mice were observed for a period of several months and their survival times and weight were recorded.

Mice receiving fetal homologous hematopoietic tissue were afforded excellent and lasting protection from the effects of the irradiation. The animals were, in fact, indistinguishable from those receiving isologous marrow. Mice inoculated with adult homologous tissue, as expected, exhibited the usual symptoms of the secondary phase reaction; such as diarrhea and extreme emaciation, leading to death. Few of the control mice survived even the initial phase of the irradiation syndrome.

Data on the body weight of animals receiving fetal hematopoietic tissue supplement the observation of increased survival time. These mice suffered no weight loss and in this respect, also, were identical to mice receiving isologous marrow.

Miss Uphoff concluded on the basis of these results that the secondary phase of the irradiation syndrome is probably the result of a graft-versus-host reaction and that this reaction can be precluded by using fetal homologous tissue inoculation.

INJECTED FOREIGN MARROW DEVELOPS INTO TUMORS IN IRRADIATED MICE

In a related study, Miss Uphoff and her colleagues observed that mice that do not die within 90 days after receiving radiation and homologous bone marrow sometimes develop tumors. They undertook to find out whether the tumors of the bloodforming organs (malignant lymphomas) originated from the cells of the host animal or the bone marrow injection.

Miss Uphoff reported that many of the mice receiving homologous marrow showed a variety of reactions, such as loss of weight, diarrhea, and occasionally dermatitis. Of the mice showing a severe reaction, only a small number survived 90 days; of those showing mild or no reaction, all survived over 90 days.

Many of the malignant lymphomas that developed in the surviving mice were tested by transplantation of bits of tumor into animals of host or donor strain. This test was based on the same principle as that used in skin grafting; a "take" occurs when an individual receives a graft of his own skin or that of an identical twin, but not when he receives a graft from another individual. In the transplantation of tumors, those that grew progressively and killed the animal were considered to be of the same strain as the animal.

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It was found that most of the tumors were of donor origin and only a few were of host origin.

The results show that the homologous bone marrow cells did not die, but survived in the irradiated host for as long as 18 months, and some developed into malignant tumors.

EFFECT OF INTERMITTENT RADIATION ON RECOVERY PROCESSES OF MICE STUDIED

Injured mechanisms for production of antibodies and leucocytes (white blood cells) apparently fail to recover during the interval between successive exposures of mice to radiation. In a study reported by Dr. Falconer Smith and his colleagues of the Radiation Branch, groups of mice were exposed to a radium source providing 2.2r or 8.8r of gamma rays per 8-hour day for 51 days, or a total of 112r or 449r, respectively. Leucocyte counts were made on the second day after completion of radiation exposure.

Immunization by a single intravenous injection of a suspension of sheep erythrocytes was done 3 days after completion of exposure, and the mice were sacrificed 5 days later, or on the eighth day, for antibody determinations in the serum.

Both antibody production and leucocyte counts were lower in irradiated mice than in controls. The values were lower for the mice receiving 449r than those receiving 112r.

Thus, recovery of the specific immune mechanism studied in the mice did not take place during the 16-hour interval which elapsed between successive exposures to as little as 2.2r per 8-hour day.

In a subsequent experiment, the scientists wished to determine whether exposure of the mice to an accumulated total of 449r would have an additive effect, if followed by an acute exposure to x-rays. Four days after exposure to 449r in the radium field, mice were exposed to 150, 250, or 350r of x-rays.

The results showed that the "chronic" exposure to 449r did not significantly alter the response of the mice to a subsequent acute exposure to x-rays.

Reports in the scientific literature suggest that prolongation of survival time of irradiated mice and rats given intermittent doses of radiation is due to recovery processes that take place between doses. Results of this study showed two of these processes -- the antibody producing capacity and leucocyte counts -- apparently did not occur under the conditions of the experiments.

NONFATAL DOSES OF S^{35} DESTROY MOUSE CARTILAGE

A collaborative study by Dr. Raymond G. Gottschalk, George Washington University School of Medicine, and Dr. Howard N. Beers, Veterans Administration Center, Martinsburg, West Va., revealed that radioactive sulfur (S^{35}) in nonfatal doses destroys cartilage cells in normal mice.

Earlier studies had shown that S^{35} has a specific affinity for cartilage cells and arrests cartilage growth. This affinity suggested a possible value of S^{35} in the treatment of chondrosarcomas (cartilaginous tumors).

Normal mice were injected with varying doses of radioactive sulfur in the form of sodium sulfate. Symptoms of radiation injury were observed by measuring food and water intake and weight of the animals and by analyzing blood samples. Histological examination indicated whether cartilage damage had occurred.

Doses above 1 millicurie per gram of animal body weight produced death by bone marrow destruction and leukopenia (destruction of white blood cells). Smaller doses, 0.5 and 1 mc per g. selectively destroyed growing cartilage cells; temporary leukopenia occurred and the animals survived.

The investigators explained the selective destruction of cartilage cells by a high uptake and long retention of radiosulfate in cartilage. In the bone marrow the uptake is relatively high, and the turnover is slower than in most tissues, although faster than in cartilage. This explains the early irreversible injury to the radiosensitive marrow cells produced by the largest doses of S^{35}.

The short range of the radiation of radioactive sulfur permits highly specific injury to the cartilage after nonfatal doses. The histological changes produced by radioactive sulfur are different from those produced by "bone seekers" such as radiostrontium.

Earlier studies indicated that the concentration of radiosulfate in chondrosarcomas generally exceeds that in normal adult cartilage, and that S^{35} concentrates in the growing portions of these tumors. The investigators suggest, therefore, that therapy may be possible with radiosulfate in advanced cases of human chondrosarcoma in which a favorable differential uptake between the normal and cancerous cells is indicated by tracer studies.

METHOD FOR DETECTING CANCER CELLS IN HUMAN WHOLE BLOOD DESCRIBED

An ideal technique for cancer detection would be a blood test that could be applied on a mass basis at reasonable cost and that would be specific enough to identify a high percentage of cancer cases at an early stage. However, before such a test can be developed, there will have to be more information on the significance of cancer cells circulating in the blood.

The preparation of the specimen to be examined is one of the crucial problems in the detection of cancer cells in the circulating blood. Scientists of the Pathologic Anatomy Branch, the Field Investigations and Demonstrations Branch, and the Surgery Branch have described a technique for preparing human whole blood so that it can be examined cytologically.

To prepare the blood for microscopic examination, it is processed by a combination of filtration, centrifugation, and addition of the enzyme, streptolysin-O, which removes erythrocytes and certain white blood cells without altering or removing tumor cells. The residual material is collected on a Millipore filter, fixed on microscope slides, stained, and cleared prior to cytologic examination. This process requires approximately two hours from the time the blood is collected to the time the slides are ready for examination.

In each of several experiments to test this technique, tumor cells obtained from a variety of sources were added to 20-ml. samples of human whole blood. In each experiment, the number of tumor cells detected was virtually identical with the number of cells originally added to the whole blood. In no case was the margin of error greater than 10 percent, and the tumor cells remained morphologically intact.

Dr. Richard A. Malmgren, Pathologic Anatomy Branch, concluded from these studies that the method described seems to fulfill all the requirements of an ideal procedure for processing samples of blood containing tumor cells for microscopic examination.

Development of this quantitative technique is an achievement of the first importance. Engell, of Copenhagen, has shown that only one-half of cancer patients whose blood contains circulating tumor cells subsequently develop metastasis. A continuing study of the significance of circulating tumor cells in individual patients representing many different anatomical types of cancer is in progress and will take at least 5 years to complete.

DETECTION OF CANCER

PROGRESS IN DEVELOPMENT OF CYTOANALYZER REPORTED

Research on procedures and techniques for the detection of cancer is designed to provide information that will permit identification of cancer in its earliest and most curable stages.

The cytologic technique developed by Papanicolaou has been studied as an aid to detection of cancer of various sites. The test involves the microscopic examination of smears containing cells shed from internal organs. The smears are fixed on slides and studied by highly trained laboratory technicians in order to detect any cancer cells that may be present. The most important use of the test has been in the detection of cancer of the uterine cervix.

Dr. John C. Pruitt, Field Investigations and Demonstrations Branch, has reported progress in studies of the cytoanalyzer, which is being developed to speed the examination of specimens obtained in the cytologic test for uterine cervical cancer.

The cytoanalyzer is an electronic instrument consisting of a scanning microscope, computer, and analyzer. Glass slides with specimens of vaginal fluid containing exfoliated cells are placed under the scanning microscope. Information is obtained on the number of cells present in the specimen and the size of their nuclei. On the basis of this information, an investigator classifies the specimen as negative, or as suspicious or positive and therefore retained for further examination.

A total of 1,095 slides was studied in the cytoanalyzer. Of these, 1,075 were known to be negative and 20 were known to be either positive or suspicious. The cytoanalyzer gave accurate results for all the positive or suspicious slides and 40 percent of the negative slides. The machine was about twice as accurate in examining slides from pre-menopausal women as from post-menopausal women. This difference is explained by the fact that non-malignant cells with large nuclei often appear in clusters in the vaginal fluid of post-menopausal women.

On the basis of these preliminary results, Dr. Pruitt concludes that the cytoanalyzer is a workable device capable of accurately selecting a significant percentage of slides that need not be examined further by cytotechnicians or pathologists. The cytoanalyzer has, therefore, the potential of being useful in the application of the cytologic test for the detection of uterine cervical cancer among large groups of women. However, a much

larger number of known positive slides will have to be examined before the study is complete.

Dr. Pruitt indicated that the successful results obtained in the study were largely due to an improved method of preparing the slides. This method permitted the cytoanalyzer to examine each cell individually.

MAIL ORDER-CYTOLOGY PROGRAM SUCCESSFUL

Results from a 10-year "mail order" cytologic screening program for uterine cancer have confirmed the value of the cytologic test and show that an effective case-finding program can be carried out by private physicians who mail specimens to a central screening laboratory.

Since 1947, the Wisconsin State Laboratory of Hygiene at Madison, under the direction of William D. Stovall, M. D., has functioned as a center for the examination of vaginal and cervical cytology specimens submitted to the Laboratory by private physicians throughout rural Wisconsin.

In 1956, the Field Investigations and Demonstrations Branch established, in cooperation with the existing State Laboratory function, a cytology research project to gather information on two main questions: 1) Can a large-scale cytologic case-finding study be carried out by mail by private physicians and clinics? 2) What is the natural history of cervical cancer?

In order to provide information on these questions, Dr. Stovall and Dr. Paul Calabresi analyzed data on 65,163 women whose cytologic specimens had been examined at the Laboratory between 1947 and 1956.

The State Laboratory of Hygiene carries out its cytology function on a "mail order" basis. Upon request, physicians receive through the mail a kit containing the necessary materials with which to obtain, package, and return to the Laboratory specimens for cytologic examination. The results of the examination are then mailed back to the physician with a recommendation for follow-up procedure.

Of all the women examined during the ten-year period, 805 had malignancies, 750 of which were detected by the cytologic examination. The remaining 55 cases represented false-negatives; that is, cases in which a negative cytologic report was followed by a positive biopsy. Of the 805 confirmed cancers, the cytologic test accurately detected 96.4 percent of the invasive cervical cancers,

94.7 percent of the in situ cervical cancers, 92.9 percent of the adenocarcinomas of the cervix, and 85.4 percent of the adenocarcinomas of the body of the uterus. Data for tumors of other sites were insufficient for statistical analysis. Approximately 75 percent of the confirmed cases of in situ cervical cancer were not clinically detectable; a reverse relationship was observed for invasive cervical cancer.

Dr. Calabresi and Dr. Stovall concluded from these findings that the cytologic test can be used as a large-scale case-finding procedure for the detection of asymptomatic cervical cancer.

Some 9,111 women received a second cytologic test at least 10 months after the first. Comparison of the occurrence rates in the first and second screenings showed a sharp decrease of both in situ and invasive cervical cancer. The rate per 1,000 women examined for in situ cervical cancer dropped from 3.2 to 1.1, and the rate for invasive cervical cancer dropped from 5.1 to 0.4. The findings confirm those reported from the Memphis, Tenn., cytologic study of some 108,000 women.

On the basis of these figures, the investigators concluded that in situ cervical cancer is an early stage in the development of invasive carcinoma of the cervix.

Finally, they suggested that a mass cytologic screening for uterine cancer is equally effective whether carried out entirely by private physicians on a mail order basis or by large, public metropolitan clinics.

CYTOLOGY USEFUL IN DETECTING URINARY CANCER

Examination of urinary sediments yields correct diagnosis (positive or negative) in 60 percent of all cases, reported a National Cancer Institute grantee at the New York Hospital, Cornell Medical Center.

Summarizing the results of a study of 2,829 cases examined during the years 1945 to 1954, Dr. N. Chandler Foot reported that this finding lends support to the theory that cytology can be useful in the early diagnosis of cancer of the genitourinary system.

All the cases reported in this study were being treated for ailments of the genitourinary system at several New York City hospitals. On the basis of final, clinical diagnosis, these patients were grouped into three categories according to the location of their disorder: 1) Bladder, ureters, and renal pelves; 2) kidney; and 3) prostate.

Cytologic study of the urine produced the most accurate results among the patients in category 1. A relatively low degree of accuracy of the cytologic test among patients in group 2 was explained by the fact that kidney tumors do not shed cells directly into urinary passages until late in the course of the disease. A similarly low degree of accuracy among patients in category 3 suggested that urine is probably not the best sample for cytologic examination and that more satisfactory results might be obtained through the use of prostatic fluid.

NEEDLE-LIKE PROBE AIDS BRAIN CANCER DETECTION BY P^{32}

Dr. Bertram Selverstone, New England Center Hospital, has reported the development of a needle-like probe, which has been found useful in detecting brain tumors made radioactive by the selective take-up of compounds containing radioactive phosphorus. In some respects the instrument resembles a Geiger-Muller counter.

The method is based on the fact that radioactive phosphorus, after injection into a vein, concentrates more in brain tumors than in normal white brain matter by a factor of nearly 20 to 1. The sensitive part of the counter is approximately 2X2X2 mm., small enough to permit the localization of radioactive phosphorus in the tissues with an accuracy approaching that of a theoretical point detector. The extent of the tumor may be mapped before, during, and after its resection by inserting the tip of the probe into suspected regions of the brain. The new probe has the following advantages over instruments previously used: a) Less penetration of the brain or tumor is required: b) demarcation of the tumor is more precise; and c) counting with the shielded sensitive tip can be done on the surface of an exposed lesion. The last-mentioned advantage is particularly useful where probing would be unsafe for the patient.

TREATMENT OF CANCER

TUMOR CELLS IN SURGICAL WOUNDS MAY BE RELATED TO LOCAL RECURRENCE

Surgery continues to be an effective means of treating cancer. However, local tumor recurrence is a frequent cause of the failure of cancer surgery. Additional confirmation of a possible relationship between the presence of cancer cells in wound washings following surgical procedures and local recurrence of the tumor has been reported by scientists of the Surgery, Pathologic Anatomy, and Field Investigations and Demonstrations Branches. The findings were summarized by Dr. Robert R. Smith, Surgery Branch.

A review of the data failed to reveal a statistically significant correlation between wound washings and such factors as type of surgery performed, patient weight loss, size of primary tumor, lymph node metastases, or degree of histological differentiation of the cancer. However, the study showed a positive correlation between the finding of cancer cells in wound washings and blood vessel invasion, and between wound washings and ulceration of the primary tumor.

FORMALDEHYDE EFFECTIVE AGAINST TUMOR CELLS IN SURGICAL WOUNDS

Indications of a possible relationship between the presence of cancer cells in wound washings and local recurrence of the tumor have suggested that a local chemotherapeutic agent capable of destroying such cells might result in a lower local wound recurrence rate. This approach is being investigated in a systematic laboratory study by the Surgery Branch. The results of the effectiveness of formaldehyde in tumor-seeded operative wounds in animals have been reported by Dr. Robert R. Smith.

Formaldehyde in dilute solutions demonstrated a striking effectiveness against tumor cells in vitro and in artifically created open and closed tissue pouches in rabbits and mice.

When formaldehyde in dilute solutions was applied at times varying from 5 minutes before to 24 hours after tumor inoculation in mice, tumor growth was prevented in 80 to 85 percent of the animals, whereas tumor growth occurred in all of the untreated controls. Treated mice developing tumor demonstrated a significant delay in time of appearance of the tumor.

The same concentration of formaldehyde placed in the wound 1 or 24 hours before tumor inoculation in mice resulted in enhanced growth of tumor and a decrease in the latent period of tumor growth.

Because the same concentration of formaldehyde that destroys tumor may, under certain conditions, increase host susceptibility to tumor implantation, any clinical application of results of these studies must be made with caution, Dr. Smith suggests.

ILEUM SERVES AS TEMPORARY URINARY BLADDER IN DOGS

The successful use of ileum as a substitute bladder in dogs has suggested clinical application to a situation in which normal urinary bladder function stops as a result of disease or surgery.

The surgical management of patients whose normal urinary flow has been disrupted as a result of disease or operative procedure or both is a clinical problem of major importance. The safety and ease of utilizing a segment of terminal ileum as a bladder conduit for the urine in patients whose autogenous bladder is either non-functioning or resected have been reported in the scientific literature. Likewise, a segment of terminal ileum has been used to replace a partially or totally destroyed or resected ureter, both experimentally and clinically.

In this study, which was reported by Dr. James R. Jude, Surgery Branch, and Dr. William J. Pieper, Endocrinology Branch, a number of female, mongrel dogs were subjected to a surgical procedure in which a bladder was constructed from a 6 to 12 cm. segment of terminal ileum. Urinary flow was diverted through this isolated bladder; the non-functioning, autogenous bladder was left intact. Studies of urine and blood chemistry carried out serially after the operation showed no complications directly related to the operative procedure, per se. Results indicated that the ileum could be utilized as an external diversion for the urine stream.

Three to eight weeks after the ileal bladder operation, a second surgical procedure was performed restoring the normal urine flow through the urinary bladder. During the short follow-up period after the second operation, all animals were either gaining or maintaining weight, blood chemistry was virtually normal, and bladder function was excellent. Further study of the dogs is in progress.

2-MEV X-RAY MACHINE GIVES GOOD RESULTS IN THERAPY OF ADVANCED LUNG CANCER

Studies in radiation therapy, as well as surgery, are designed to produce new knowledge and to refine techniques in order to achieve ever increasing prolongation of the lives of cancer patients. Dr. Ruth J. Guttman, College of Physicians and Surgeons, Columbia University, reported

on the results of radiation therapy in 144 patients with advanced cancer of the lung, which had spread to adjacent organs and lymph nodes. Treatment with a 2-million electron volt roentgen ray machine resulted in the survival of one-third of the patients for more than a year. Four patients survived longer than 4 years. Observations made in the course of this study were: evidence of greater response of malignant lesions of lining tissue (epithelium) compared with those of glandular tissue and microscopic proof that it is possible to destroy even a large carcinoma of the lung with external radiation.

NEW TECHNIQUE PERMITS IMPROVED MEASUREMENT OF RADIATION DOSAGE

The amount of radiation delivered to internal body structures may be determined by measuring changes in the acidity of a gel containing chloral hydrate, Dr.Howard L. Andrews, Radiation Branch, has reported.

Development of a chemical technique for depth-dose measurements was undertaken in an effort to improve techniques of administering radiation.

This method is based on the fact that X-rays produce hydro chloric acid from a water solution of chloral hydrate. In the technique described, chloral hydrate is added to a hot agar gel. As it cools, the gel can be molded to any desired configuration and thus be made to represent the internal structures in which the radiation dose is to be measured.

The amount of hydrochloric acid produced in the gel dosimeter can be measured by spectrophotometry or by electrical conductivity. The addition of an indicator dye permits visualization of the color change resulting from changes in acidity of the gel.

LABORATORY ASSAY OF 38 ANTILEUKEMIA DRUGS SHOWS METHOTREXATE MOST ACTIVE

Chemotherapy is a valuable adjunct to surgery and radiation because of its effectiveness in temporarily halting the progress of certain cancers and in increasing the well-being of cancer patients. Surgery and radiation are frequently not effective against cancer that has spread from the original site to other parts of the body, or against generalized cancers, such as cancers of the blood-forming organs. The hope of many scientists is eventually to find compounds or drugs

that will selectively seek out and destroy cancer cells and tissues without harming the surrounding normal, healthy cells.

Dr. Abraham Goldin, Laboratory of Chemical Pharmacology, reported the comparison of the anticancer effectiveness of compounds tested by an assay procedure developed previously in collaboration with the Biometry Branch. Of 38 antileukemia drugs tested in mice, methotrexate produced the greatest increase in survival. The next most effective drug was the tranquilizing agent, reserpine.

The assay procedure involves the continued daily treatment of leukemic mice at a series of dosage levels of the drug. Treatment is begun when the local tumor at the site of leukemic inoculation is approximately 10 millimeters in diameter. At this time, the disease is systemic as well as local, and untreated animals die 2 to 3 days later.

The antileukemic effectiveness of a drug is dependent upon the specificity of its action as shown by an increase in survival time of the animal. If a dose possesses antileukemic specificity, survival time increases with increasing dosage until a level is reached at which toxicity of the drug for the host becomes dominant. Further increases in dosage result in decreasing survival time.

In each of 14 experiments, the anticancer drug methotrexate (amethopterin), which is used clinically, was used as a reference compound and was compared with several other compounds. When treatment was begun 7-10 days after inoculation of the tumors, mice receiving methotrexate survived an average of 12 to 19.5 days longer, while untreated controls survived an average of 1 to 4 days longer.

Reserpine was found to be 62 percent as effective as methotrexate. The purine antagonists, 6-mercaptopurine, thioguanine, and 8-azaguanine, all ranged from 54 to 57 percent as effective as methotrexate. The other compounds tested, when compared with methotrexate, ranged downward in activity from 52 to zero percent.

These results confirm the usefulness of this assay procedure and suggest that it may be adaptable as a screening tool in the evaluation of drugs.

METHOTREXATE DERIVATIVE FOUND MORE ACTIVE THAN PARENT COMPOUND

In another study, Dr. Goldin and his colleagues reported that a derivative of methotrexate (amethopterin), when evaluated by the same assay procedure, was substantially more effective than methotrexate in increasing the survival time of mice with advanced leukemia.

The purified drug, 3' , 5' - dichloroamethopterin, produced a 75 percent increase in survival time beyond that achieved with methotrexate. The optimal dose of dichloramethopterin was approximately 50 times that of methotrexate and produced survival times beyond 50 days in a few mice.

Results of these experiments emphasize the need of such systematic studies of compounds closely related to drugs of known activity. Additional derivatives of methotrexate and related compounds are being studied by the comparative testing procedure developed in this Laboratory.

Dichloroamethopterin has been placed in preliminary clinical trial under the program of the Cancer Chemotherapy National Service Center.

SYSTEMATIC RESEARCH YIELDS PROMISING ANTICANCER DRUG

Promising results have been obtained in mice with a new drug, azauridine (AzUR), reported Dr. Julian J. Jaffe and his colleagues at the Yale University School of Medicine. The drug was selected on the basis of theoretical considerations. It is related chemically to 6 - azauracil (AzU), which was synthesized by these investigators in 1955 and was used with encouraging results for the treatment of cancer in human patients by National Cancer Institute scientists.

Studies with these substances are based on the assumption that anticancer chemicals act by interfering with life processes (metabolism) of cancer cells. Accordingly, it appeared reasonable to assume that significant interference with the formation or utilization of one important cell component-uracil--could profoundly affect the reproduction of cells. This line of reasoning led to the synthesis and anticancer tests of AzU, an antagonist of uracil.

Later studies suggested that inhibition of cell reproduction in bacteria appears to require the formation of ribose-containing derivatives of AzU. Tests were than made of the urine of mice receiving AzU and the presence of material behaving like AzU-riboside (AzUR) was demonstrated. These findings suggested the use of AzUR as an anticancer drug.

In the present study, comparison was made of the activity of AzUR and AzU as inhibitors of the growth of three mouse tumors. Treatment was begun 24 hours after introduction of the cancer cells or tissue and was given either intraperitoneally at intervals of 8 hours or by way of the drinking water. Therapy was terminated after 6 or 7 days, after which the mice were sacrificed. The weights of tumors were then compared with those of the tumors of untreated control mice.

The results showed that AzUR is significantly more effective than AzU in inhibiting the growth of the mouse tumors, and when compared on a molar basis (weight of substance per weight of 1 molecule of substance), the carcinostatic activity of the riboside is 10 to 20 times that of AzU.

Dr. Jaffe reported: "Since the ability of individual animals to form active derivatives of AzU may be variable, and may be influenced by dietary or many other factors, it would seem preferable now to give attention to the possible applications of AzUR as a carcinostatic agent, particularly in man."

TSPA MODERATELY EFFECTIVE IN RETARDING LOCAL GROWTH OF IMPLANTED MOUSE TUMORS

In a study reported by Dr. William M. Kramer of the Surgery Branch, systemic administration of the drug triethylenethiophosphoramide (TSPA) shortly after tumor inoculation was somewhat effective in retarding local tumor growth in mice.

In one experiment, mice received injections of S91 melanoma tumor cells intramuscularly into a hind leg. Animals to be treated were given 10 micrograms of TSPA per gram of body weight intraperitoneally at specific times after tumor inoculation. Control mice received either a saline solution or no treatment. When all tumors were large enough to be measured, the average diameter of treated and control tumors was determined. The intramuscular S91 tumors that developed in mice treated with TSPA at the time of inoculation or within two days were smaller than the control tumors.

In another experiment, mice received inoculations of S91 melanoma tumor cells intraperitoneally, and were treated with TSPA according to a schedule similar to that used in the previous experiment. Twenty-one days later the mice were killed and their peritoneal cavities examined. The number and size of peritoneal S91 melanoma tumors developing in mice treated with TSPA were smaller than those in the control mice.

The studies showed that TSPA has little or no effect against sarcoma 37 or Krebs 2 carcinoma, tested under the same conditions as the S91 melanoma. Furthermore, susceptibility of human tumor cells to TSPA remains a matter of conjecture.

The authors concluded their report with a comment that caution is necessary in extrapolating data from animal tumor experiments to human experience.

MULTICOMPOUND CHEMOTHERAPY PRODUCES MARKED REGRESSION OF BREAST CANCER IN MICE

In addition to seeking new, powerful chemicals for the treatment of cancer, science is attempting to develop improved ways of using the agents that are already known to possess some degree of anticancer activity. In one study of this kind reported by Dr. Daniel M. Shapiro, College of Physicians and Surgeons, Columbia University, four agents used simultaneously caused marked regression of breast cancer of mice, whereas lesser combinations, regardless of dosage, produced only retardation or cessation of tumor growth.

The four compounds used in this study were nicotinamide, 8-azaguanine, deoxpyridoxine, and testosterone. When they were tried singly, or in any combination of three, the best result obtained was a stoppage of tumor growth. It took all four compounds used together to produce actual regression in the size of tumors.

In general, administration of the four compounds produced occasional deaths and a host weight loss of six to nine percent. When treatment was extended beyond six to eight days in an attempt to produce complete regressions, serious host toxicity developed.

These findings support the principle of simultaneously attacking tumor by a variety of metabolic pathways as one means of improving the effectiveness of known anticancer agents. The author suggests that selectively increasing the number of agents simultaneously administered can progressively augment anticancer activity and possibly produce complete tumor eradication.

AET PROTECTS MICE AGAINST LETHAL DOSE OF NITROGEN MUSTARD

A chemical, AET, protects mice against the toxic effects of nitrogen mustard, an anticancer drug used in the treatment of Hodgkin's disease, Dr. Margaret G. Kelly, General Medicine Branch, reported. Tumor-bearing mice pretreated with the chemical, aminoethylisothiuronium bromide hydrobromide (AET), were completely protected against a dose of nitrogen mustard that would ordinarily kill 50 percent of the animals. In no case was the anticancer effect of nitrogen mustard suppressed by AET.

The investigation stemmed from reports from the Oak Ridge National Laboratory of the Atomic Energy Commission that AET was effective in protecting mice and monkeys against the lethal effects of radiation. These observations suggested the possibility that AET might protect against the toxic effects of alkylating agents, such

as nitrogen mustard. This is a "radiomimetic" drug, producing in tissues effects similar to those produced by radiation.

The tests were made on mice that had a variety of transplanted tumors, including leukemia, lymphoma, sarcoma, and lymphosarcoma. Injection of AET alone produced no demonstrable effect on any of the tumors, but pretreatment with AET permitted the use of higher doses of nitrogen mustard.

The mean survival time of mice bearing implants of leukemia (L1210) and sarcoma (reticulum-cell sarcoma No. 6867) was always longer in animals pretreated with AET than in mice given high dose levels of nitrogen mustard only, and was increased 20 to 50 percent over that of untreated controls.

In mice bearing subcutaneous implants of either of these two tumors, nitrogen mustard delayed infiltration of the liver with tumor cells. This action of the drug was also not inhibited by pretreatment with AET.

Histopathologic studies on mice pretreated with AET indicated that it decreased but did not entirely prevent the toxic effects of nitrogen mustard in bone marrow and intestine of normal and tumor-bearing mice.

AET showed a weak protective effect against the toxicity of thio-TEPA, but none against other "radiomimetic" drugs, such as Myleran, colchicine, and trimethyl colchicinic acid.

RADIOACTIVE ISOTOPES AID BASIC CHEMOTHERAPY STUDY

In cancer chemotherapy research, important chemical constituents of cells are studied. Previously reported studies, in which scientists of the Laboratory of Chemical Pharmacology collaborated, showed that injection of nicotinamide, a B-complex vitamin, into mice produced an 8- to 10-fold increase in the diphosphopyridine nucleotide (DPN) content of the liver. DPN is a substance necessary for cell respiration and growth. The mechanism by which nicotinamide stimulates DPN synthesis is under study by these scientists, as part of an extensive program to develop a new scientific basis for designing chemotherapeutic agents against cancer.

The DPN molecule consists essentially of two mononucleotides, nicotinamide mononucleotide(NMN)and adenylic acid (AMP). Each mononucleotide consists of three components: NMN contains the

nitrogenous base, nicotinamide, the 5-carbon sugar, ribose, and phosphoric acid; AMP contains the nitrogenous base, adenine, ribose, and phosphoric acid.

Dr. Louis Shuster has reported recent results obtained by the use of labeled precursors, C^{14}-glucose and C^{14}-ribose, in studying the formation in vivo of the various constituents of the DPN molecule.

In one study, mice were injected intraperitoneally with various doses of nicotinamide and then with labeled glucose (6-carbon sugar) or ribose. The DPN was obtained from the livers of the mice and its concentration was measured; the component mononucleotides were isolated and measured after the DPN molecule was broken by hydrolysis. In the second study, the mononucleotides were obtained in a similar manner and broken down further to their component parts.

Results of the studies showed that 1) nicotinamide appears to stimulate a greater production of the AMP portion of the DPN molecule than the NMN component; 2) the specific activity of DPN and free adenylic acid increased with increasing dosage of nicotinamide to a level of 500 mg. per kilo of animal body weight; 3) considerable quantities of ribose-labeled DPN were obtained by injecting mice with nicotinamide and C^{14}-labeled glucose, indicating that glucose was converted to ribose; and 4) the conversion of glucose to ribose appears to be accomplished by way of the transaldolasetransketolase pathway rather than by oxidative decarboxylation.

This research points up once again some of the problems confronted in the antimetabolite approach to cancer chemotherapy, especially the ease with which important large molecules are formed from common and abundant precursors.

ACTION OF, AND RESISTANCE TO, CORTISONE TREATMENT STUDIED IN ANIMALS

Other studies in chemotherapy research produce information on the mechanism of action of anticancer agents. Although cortisone is widely used in the management of acute leukemia, its effect is only temporary and the mechanism of its action is still not known. To obtain better understanding of the drug's action, scientists of the Laboratory of Biology are studying the effects of cortisone and the development of resistance to cortisone.

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Miss Julia Lampkin reported a study in which cortisone induced regression of a mouse lymphosarcoma, a type of lymphoid neoplasm that originated in the thymus of a hormone-treated male mouse. Bits of this tumor were inoculated subcutaneously into mice for the initiation of a subcutaneous line and intraperitoneally into other mice for the initiation of an ascites tumor-cell line.

The results showed that the lymphosarcoma was highly sensitive to cortisone through 12 transfer generations. When cortisone was given either immediately following tumor inoculation or after the tumor had achieved a considerable volume, it prolonged the life of animals bearing the subcutaneaous neoplasm. Under some conditions of treatment with cortisone, mice in the second transfer generation did not develop progressively growing tumors and they survived for almost a year.

Cortisone induced virtually complete regression of established subcutaneous tumors in 3 to 6 days; however, following regression, the tumors reappeared and eventually killed the mice. An ascites tumor-cell line that was developed responded in the same manner as the established subcutaneous graft by undergoing profound regression following administration of cortisone.

Resistance of the tumor to cortisone was developed by inducing incomplete regressions and by allowing regrowth to occur each time before administration of cortisone. Methotrexate profoundly inhibited the growth of the resistant as well as the sensitive line of the neoplasm. The cortisone-sensitive line was also very sensitive to a related steroid, prednisone, but the cortisone-resistant line showed a lack of response to prednisone, indicating cross resistance.

The investigator concluded that further study along these lines would be profitable. The process of the development of cortisone resistance needs further clarification and is being studied in this laboratory. Miss Lampkin also suggests that the lymphosarcoma of the mouse may be useful for the study of the antileukemic action of compounds related to cortisone.

MOUSE STUDY SUGGESTS ZYMOSAN STIMULATES RESISTANCE TO CANCER

A group of investigators at the Sloan-Kettering Institute for Cancer Research reported preliminary results a year ago on the action of zymosan, a yeast product, in tumor-bearing mice. They reported extrusion of tumors in a high percentage of treated animals, and suggested that zymosan exerts its effect by

stimulating the animals' natural defense processes against cancer. The investigators have now published detailed results which confirm their earlier conclusion.

In the study, reported by Dr. William T. Bradner, mice bearing a transplantable tumor, Sarcoma-180, received various doses of zymosan. The investigators found that low doses of the drug were more effective than high doses, which is contrary to the typical dose-response reaction for anticancer drugs in dose ranges relatively innocuous to the host. When mice whose tumors were destroyed following zymosan administration received a second transplant of Sarcoma-180, the tumors failed to grow in every case. In addition, tissue culture studies showed that zymosan had no direct effect on tumor cells in vitro.

Dr. Bradner concludes from these observations that the anticancer effect of zymosan is entirely an immune response mediated by the host. He suggests that zymosan may influence some intermediate factor, perhaps a function of the properdin system, that is related to resistance to cancer.

DIFFERENT METABOLISM MAKES ANTICANCER DRUG ACTIVE IN RATS BUT NOT IN MAN

The scientific literature contains numerous reports of regression of established tumors in mice and rats following administration of riboflavin deficient diets and/or riboflavin antagonists. It is difficult to induce clinical riboflavin deficiency in man by dietary restriction of this vitamin. Furthermore, analogues available to date have been incapable of inducing signs of riboflavin deficiency or evidence of tumor regression in cancer patients.

A report of tests with a newly-synthesized riboflavin antagonist, 6, 7-dimethyl-9-(2'-acetoxyethyl)-isoalloxazine, (U-2112), showed that the drug produced partial to complete regression of established tumors in rats. In view of its anticancer effect against rat tumor growth and low toxicity in rats and dogs, U-2112 was selected for clinical trial by scientists of the General Medicine Branch.

Dr. Montague Lane reported that ten patients with several types of advanced neoplasms received oral doses of U-2112 on varying regimens. None of the patients showed clinical improvement or objective evidence of tumor regression, nor did any show riboflavin deficiency or drug toxicity. The metabolism of U-2112 was found to be different from that in the rat; a significant percentage of the administered drug was excreted in the urine unaltered by the rat, whereas hydrolysis of the drug was practically complete in man. The

investigators attribute the failure to produce riboflavin deficiency and the lack of therapeutic effect to a species difference between rats and humans in the metabolism of U-2112.

PROMISING RESULTS ACHIEVED IN CLINICAL TRIAL OF NEW ANTICANCER DRUG, 5-FU

Investigators at the University of Wisconsin Medical School have studied the clinical effects of 5-fluorouracil (5-FU), an anticancer agent they first described about one year ago. Dr. A.R. Curreri has now reported the results of the tests in 103 patients afflicted with far advanced cancer not amenable to surgery, x-ray, or hormonal therapy.

In thirty-five patients receiving an adequate therapeutic dose of 5-FU, nine patients (approximately 25 percent) showed objective regression of solid tumors. The drugs produced marked toxic reactions, however. Among the types of cancer that responded to 5-FU were tumors of the breast, liver, rectum, and other sites. The majority of the patients treated reported subjective improvement and decrease of pain to such an extent that analgesics were no longer required.

In establishing optimum dose level for 5-FU, the investigators observed that the drug has to be given at doses near the toxic level to produce the best results. This phenomenon is similar to that observed when the antifolics are given. Patients receiving less than 15 milligrams of 5-FU per kilogram of body weight per day for five days showed relatively few toxic reactions, and virtually no tumor regression. The majority of the patients who received 15 mg/kg/day for five days or more developed nausea, vomiting, and diarrhea. Several patients also experienced excessive loss of hair. Bone marrow depression was another frequent manifestation of toxicity. Tumor regression was noted only in those patients manifesting severe toxicity.

The effects of 5-FU toxicity are of relatively short duration once the drug is withdrawn. Thus far in patients with solid tumors who have responded to repeated courses of 5-FU, no development of resistance to the drug has been observed. The investigators suggest that clinical studies with 5-fluorouracil should be continued and extended.

5-FU DERIVATIVES FOUND MORE ACTIVE THAN PARENT DRUG IN ANIMAL STUDIES

Laboratory studies of the tumor-inhibiting properties of two compounds related to 5-fluorouracil (5-FU), namely 5-fluorouridine and 5-fluoro-2-deoxyuridine, were reported by Dr. Charles Heidelberger, University of Wisconsin Medical School. The latter two compounds had significant tumor-inhibitory activity against a mouse leukemia and a solid tumor, adenocarcinoma 755. In the Ehrlich ascites carcinoma, against which 5-FU was effective in producing a 420 percent increase in survival time of mice, both uridine compounds were more effective than 5-FU.

In mice, the toxicity of the three drugs, in descending order, was 5-fluorouridine, 5-FU, and 5-fluoro-2-deoxyuridine. In rats, the toxicity of 5-fluorouridine was much less pronounced than in mice, and the compound was very effective in the treatment of a liver cancer, the Novikoff hepatoma.

As a result of the promising results obtained in laboratory studies, 5-fluouro-2-deoxyuridine has been placed in clinical trials under the program of the Cancer Chemotherapy National Service Center.

SPINAL METHOTREXATE DOSE CONTROLS NEUROLOGICAL SYMPTOMS OF LEUKEMIA

When leukemia cells penetrate the central nervous system, a leukemic child may develop one or more of a variety of neurological manifestations of the disease, ranging from lethargy and headache to paralysis, blindness, and coma. Such symptoms often appear in the child whose bone marrow shows no sign of the disease. This suggests that the antileukemia agents now in clinical use are relatively ineffective against leukemia cells in the central nervous system. Radiation therapy can be used to control neurological manifestations of leukemia. But the effects of this treatment are only temporary, and many radiologists are opposed to administering X-rays to the brain and spinal cord to gain short-term improvement.

With this background as a starting point, a group of investigators at the Memorial Center for Cancer and Allied Diseases, Sloan-Kettering Institute for Cancer Research and the Department of Medicine, Cornell University Medical College, sought to find better means of controlling the neurological symptoms associated with leukemia. Dr. James A. Whiteside has reported the results of a study of the effect of methotrexate injected into the cerebro-spinal fluid.

Primary studies in dogs showed that methotrexate could be injected directly into the spinal fluid without causing serious host toxicity. Within minutes after the injection, high concentrations of the drug were detected in the spinal fluid.

The investigators then administered methotrexate to 5 leukemic children who exhibited a variety of neurological manifestations. The children tolerated the drug well, and all showed high concentrations of methotrexate in the cerebrospinal fluid within minutes after administration.

All 5 patients experienced clinical improvement of neurological symptoms. Two of the patients who had become paralyzed due to spread of the disease to the central nervous system regained the use of limbs and extremities. Another patient, blind as a result of neurological involvement of leukemia, regained her sight and was able to play actively. Two patients who were considered refractory to methotrexate showed favorable neurological response after intraspinal drug therapy.

Improvement in neurological symptoms usually occurred in 7 to 14 days following treatment and lasted for about 6 weeks. In all cases, repeated administration of methotrexate was effective in controlling neurological symptoms when they reappeared. Because all the children were still living at the time the work was reported, it was impossible to determine the exact location and extent of lesions in the central nervous system.

Dr. Whiteside concluded from these studies that the control of neurological symptoms in leukemia patients by intraspinal injection of antileukemia drugs may become a valuable tool in the hands of the clinician who is faced with the problem of providing total care for the leukemic child.

PREDNISONE, PREDNISOLONE ARE AS EFFECTIVE AS 6-MP IN ADULT ACUTE LEUKEMIA

Prednisone and prednisolone have been found as effective as 6-mercaptopurine against acute leukemia in adults, according to scientists at New England Center Hospital and the Tufts University School of Medicine, Massachusetts. These new drugs are adrenal steroids and are chemically related to cortisone which is used clinically in the palliative treatment of acute leukemia.

Dr. Norma B. Granville reported that prednisone and prednisolone were used interchangeably and given orally in large doses (250 mg. or 1000 mg. per day) to 20 adult patients for a period

of two weeks, after which the dosage was reduced to a maintenance level (5 to 50 mg. per day). Side reactions were more frequent and more serious in the 1000 mg. group.

In most patients remarkable symptomatic improvement was evident within the first 24 to 48 hours of treatment. Five complete (clinical, hematologic, and bone marrow) and two partial remissions occurred. At the time of the report, one patient was alive and well 24 months after the start of therapy. The other periods of complete remission ranged from 3 to 9 months.

Dr. Granville said that the results could not be unequivocally attributed to the effects of prednisone or prednisolone alone, because many of the patients received other drugs before and after the completion of the initial two week course. However, she concluded that massive doses of prednisone are at least as effective as 6-mercaptopurine and, in the 250 mg. dosage, less injurious.

The results of the study are in agreement with results previously reported in the scientific literature.

REPORT FURTHER RESULTS ON CHORIOCARCINOMA TREATED BY METHOTREXATE

Dr. Roy Hertz, Endocrinology Branch, has reported further results on the treatment of choriocarcinoma and related trophoblastic tumors with the drug, methotrexate, given by a specially devised regimen. Choriocarcinoma is a rare but highly malignant tumor of embryonic origin. In women it arises in the uterus during or after pregnancy. In men the disease appears as a testicular tumor.

Dr. Hertz described the findings in 27 women treated during a period of 2 1/2 years. All but five of the patients were gravely ill on admission to the Clinical Center. Most of them showed lung metastases, and a few presented evidence of central nervous system metastases.

Response to treatment was measured by the amount of the hormone, chorionic gonadotropin, produced by the tumor and excreted in the urine, as well as by chest X-ray changes, and improvement in pelvic and cerebral involvement.

Complete remissions with no hormonal, radiologic, or physical evidence of recurrence for periods of 8 to 29 months were observed in 5 patients. Eleven patients showed remission with persistent manifestation of disease, and the remaining 11 patients died either after initial remission or during initial therapy without response.

None of the five male patients exhibited a significant response to identical forms of therapy.

Dr. Hertz concluded that intensive methotrexate therapy is a practicable and highly useful form of medical management of advanced choriocarcinoma and related trophoblastic disease of women. However, the relationship of the therapy to the entire clinical course still remains to be fully evaluated.

Dr. James F. Holland has reported the apparent suppression of choriocarcinoma in a patient treated with methotrexate. This is believed to be the first case of its kind other than those reported by Dr. Min C. Li, Donald B. Spencer, Dr. Roy Hertz, and Dr. Herbert A. Lubs, of the National Cancer Institute's Endocrinology Branch.

A 33-year old woman was treated according to the schedule prescribed by Dr. Li, who is now on the staff of the Memorial Center for Cancer and Allied Diseases. The patient, who was first placed on drug therapy in the fall of 1956, responded almost immediately. The level of the hormone, chorionic gonadotropin, fell sharply to normal after the first course of methotrexate. Metastatic lesions in the lungs began a progressive decrease in size, as did the primary pelvic tumor. By February 1957, the patient was apparently completely free of any malignant growth.

At the time of the report, the patient was in remarkably good health. Dr. Holland plans to administer methotrexate at 30- to 60-day intervals, depending on toxicity, to maintain suppression of the tumor if any remains. Toxic side reactions were severe at times in this patient but never life-threatening, Dr. Holland reported.

Items of Interest on Research Studies Conducted and

Supported by the National Heart Institute

ATHEROSCLEROSIS AND CORONARY HEART DISEASE

STEROL AND UNSATURATED OIL MAY LOWER CHOLESTEROL BY DIFFERENT MECHANISMS

Two major dietary factors from vegetables have received particular research attention because they lower blood lipids and because elevations of blood lipids are associated with atherosclerosis. These factors are the plant sterols and the unsaturated vegetable fats. Authorities disagree on the identity of the constituents in the vegetable oils which lower blood cholesterol, some holding that it is simply their sterol content, others maintaining that the unsaturated fatty acid content is the critical factor.

Pointing this out in the journal Circulation, Drs. John Farquhar and Maurice Sokolow of the University of California Medical School report the results of a long-term investigation of the effects of the most common plant sterol, beta-sitosterol, and an unsaturated vegetable fat, safflower oil, on the serum lipids of humans.

Fifteen subjects with clinical evidence of atherosclerosis were studied during a seven-phase experiment lasting 35 to 54 weeks. They were given, in random sequence, beta-sitosterol (18 grams daily), safflower oil (81 grams daily), and a combination of the two. Three experimental periods of the study were alternated with four control periods during which placebos replaced the sterol and oil. During the experimental periods, the oil was substituted, calorie for calorie, for the saturated fat in the control diet. This maintained the subjects at constant weights.

The NHI grant-aided investigators report that a rapid and sustained fall in serum cholesterol levels resulted in all subjects from both agents. This fall was limited to the low-density, or beta, fraction of their lipoproteins--the cholesterol level of high density alpha fraction remained unchanged. The average drop in beta lipoprotein

cholesterol was 22% for each agent. When used in combination, however, the average drop was 34%. The drop in cholesterol was a little slower in response to the oil than to the sterol.

"The somewhat slower change in beta lipoprotein cholesterol levels during and after administration of safflower oil than during and after beta-sitosterol suggests that the two compounds differ in mechanism of action," the investigators state. "It seems very unlikely that the 50% greater decrease in serum cholesterol effected by the two agents combined results from the added action of the small amount of sitosterol present in the safflower oil..."

The investigators point out that the effect of the safflower oil might be ascribed to its high unsaturated (linoleic) fatty acid content. They admit that it might also have been due simply to the omission of animal and otherwise saturated fats from the diet when the oil was used. However, the serum cholesterol rose in two subjects on safflower oil when the oil was saturated by chemical hydrogenation to destroy its linoleic acid.

"The uniformity of serum lipid response to the test agents, coupled with the stability of average body weights and control beta-lipoprotein cholesterol concentrations, indicates that confidence can be placed in the results of long-term dietary studies using nonformula diets in well-instructed ambulatory subjects," the investigators summarize.

ESTROGENS AND THYROID ACT TOGETHER TO PROTECT AGAINST ATHEROSCLEROSIS

The protection that female sex hormones (estrogens) provide against cholesterol-induced atherosclerosis in the chick appears, from NHI grant-supported studies in Chicago, to depend on the presence of a normally functioning thyroid gland.

Drs. Ruth Pick, Jeremiah Stamler, and Louis N. Katz of Michael Reese Hospital rendered groups of chicks hypothyroid by destroying their thyroid glands or by administering thiouracil, a drug which interferes with the production of thyroid hormone. Throughout the experiment all chicks, including control groups with normal thyroid function, were maintained on a 1 or 2 percent cholesterol diet, which is known to produce coronary and aortic atherosclerosis in normal chickens.

All groups of hypothyroid chickens showed markedly higher blood cholesterol than the controls. This tended to be associated with intensification of aortic and coronary atherogenesis, the investigators report. In hypothyroid chicks treated with estrogens, which are known to inhibit cholesterol induced coronary atherosclerosis, the thyroid deficiency grossly counteracted the ability of the estrogens to prevent coronary atherosclerosis.

Thyroid and estrogenic hormones apparently act synergistically to render the coronary vessels resistant to plaque formation, the investigators conclude in their report in the journal Circulation Research. The experiment demonstrates for the first time a counteraction of estrogen prophylaxis, they point out. This contrasts with previous experiments in which excess adrenocortical and male sex hormones, and deficiency of pancreatic activity failed to influence the protective effect of estrogens on coronary atherogenesis.

CLOT-DISSOLVING PLASMIN EFFECTIVE AGAINST CLOTS UNDER THREE DAYS OLD

The value of using the body's own clot-dissolving agent, plasmin, to remove obstructing blood clots from the circulation appears from studies at the Roswell Park Memorial Institute and the University of Buffalo Medical School to be limited largely to clots less than three days old.

The investigators, Drs. Nathan Back, Julian Ambrus, C. Lenore Simpson, and Sidney Shulman, found in dog studies that clots older than three days did not yield to repeated injections of human plasmin. They observed that after three days the clots became separated from the blood stream and from the action of the plasmin by an enveloping layer of endothelium, the tissue that lines blood vessels.

The investigators used the bacterial enzyme, streptokinase, to activate preparations of human plasmin, which they then injected into the dogs. Although plasmin is a natural constituent of blood, very little of it is normally found there in its active form. It occurs mostly as an inactive precursor called plasminogen. Streptokinase is the best presently available "activator" for converting inactive plasminogen into clot-dissolving plasmin.

"Five daily treatments with 30 plasmin units per kilogram of human plasmin completely lysed clots less than three days old, but did not affect those older than three days," the investigators conclude. "Histologic examination revealed that three days after formation, the clot is already largely separated from the blood stream by a layer of endothelial cells which may prevent plasmin from reaching the clot. Increased clot density may also be a factor in the clot becoming resistant to treatment."

Results of these NHI grant-aided studies are reported in detail in The Journal of Clinical Investigation.

STRESS OF EXAMINATION WEEK RAISES STUDENTS' BLOOD CHOLESTEROL LEVELS

A significant rise in serum cholesterol accompanied the mental and emotional stress of examination week in 44 medical students studied by P. T. Wertlake, A. A. Wilcox, M. I. Haley, and J. E. Peterson at the College of Medical Evangelists in Los Angeles. The average serum cholesterol level of the students rose eleven percent during the four-day examination period, the National Heart Institute grant-aided study showed.

"As one might expect, these individuals vary greatly in their clinical and biochemical responses to stress", the investigators report in Proceedings of the Society for Experimental Biology and Medicine. Twenty of the subjects showed increases ranging from 16 to 137 milligrams percent over a mean control level of 213. Ten showed increases ranging from 10 to 15 mg. percent over their control averages of 223, and fourteen showed increases of 9 or less over 216 for the three-day control period.

Some individuals experienced a remarkable increase in their serum cholesterol, the investigators report. Particularly noted was one student whose control sample average of 259 mg. percent increased to 395 during the four examination days. On two of these days his serum cholesterol reached levels of 520 and 536.

"It is apparent that the situational stress of examination week must have had different meaning, and hence a differing stress value for the subjects", the investigators noted. "Further investigation is needed to establish whether this increase is harmful or simply part of a normal and desirable bodily adjustment to mental and emotional stress".

OCCLUSION TECHNIQUE FOR VISUALIZING CORONARY ARTERIES SHOWS PROMISE

A practical method for visualizing the coronary circulation has been developed by Drs. Charles Dotter and Louis Frische in the NHI grant-aided research at the University of Oregon. The technique, which utilizes a special balloon-bearing catheter, is called "occlusion aortography".

From the tip of the special catheter, which is introduced through an arm artery, an X-ray contrast medium is injected within the base of the aorta at the point where the two main coronaries branch out over the heart muscle. The balloon, inflated near the catheter tip just before the injection is made, blocks the aorta completely, diverting all of the blood and contrast substance into the coronaries while rapid serial X-ray pictures are taken. Arteriosclerotic blockage, reducing blood supply to portions of the heart muscle, is thus clearly revealed by the concentrated opaque contrast medium flowing

through the coronaries, Drs. Dotter and Frische report in the journal Radiology.

Commenting on the national problem of arteriosclerotic heart disease, they write, "We believe that a direct surgical attack constitutes the most logical approach to this essentially mechanical obstruction. If so, it will be necessary to obtain detailed anatomical information concerning the extent and location of disease in order to select patients for surgery, plan the operative approach, and evaluate the results."

In patients with coronary disease, the investigators inject 10 or 15 cubic centimeters of nitrous oxide gas into the balloon via one of the catheter's twin passageways. The balloon is thus inflated, occluding the aorta completely, for 5 to 10 seconds. Drs. Dotter and Frische are assured that blocking all blood flow (except coronary) from the heart for this period of time is without significant hazard in patients with healed myocardial infarcts. Among the reasons for their assurance is their experience with 500 completely harmless aortic occlusions for up to twenty times this duration in preliminary dog experiments, many involving dogs with a ligated coronary artery.

The investigators are also experimenting in dogs with the technique of stopping the heart beat with a prior injection of acetylcholine from the catheter to further improve coronary visualization. Injection of a very small amount of this neurohormone into the coronaries stops the heart harmlessly, because the body's natural mechanisms for inactivating acetylcholine work so swiftly. "So rapid is the rate of inactivation of acetylcholine within the myocardium that virtually no active drug escapes into the circulation to cause undesirable side effects", they explain. "Acetylcholine can be said to have a built-in safety factor". The heart simply hesitates for a few seconds--long enough for the injection and X-rays--and then resumes beating. Aortic occlusion allows the use of much less acetylcholine than would be necessary without occlusion, because all of it can be directed into the coronaries, the Portland workers explain. Describing their dog experiments with cardiac arrest, they report, "In all cases, the arrest was spontaneously terminated within 15 seconds of its onset. No abnormal rhythms were encountered and normal sinus rhythm was present a few seconds after the first returning beat following arrest."

A by-product of the use of occlusion aortography to visualize coronaries was the discovery that occlusion with the balloon catheter also permits remarkably detailed X-ray visualization of the descending aorta itself, beyond the inflated balloon. Conventional arteriography often fails, the investigators explain, because the contrast substance

is swept away by the great speed of the blood which flows at rates up to 150 cm. (60 inches) per second. With their technique of "postocclusion" arteriography, the artery lumen is photographed in a static, half-empty state reflecting its intraluminal pressure and volume and at a reduced size which may itself be revealing.

"In summary," they write, "the use of occlusion catheters for selectively excluding or gaining access to various portions of the cardiovascular system promises to have considerable future impact on experimental and clinical medicine."

DIET STUDIES IN CHICKENS STRESS FACTORS OTHER THAN DIETARY FATS

Findings suggesting the importance of adequate protein in protecting against diet-induced atherosclerosis are reported from NHI-supported studies in chickens at the University of Illinois. Results of the studies also indicate that there are exceptions to the rule that lowering blood cholesterol lessens atherosclerosis--some cholesterol-lowering diets may actually aggravate this disease, at least under experimental conditions.

Investigating the effects of dietary protein in blood cholesterol, lipoproteins, and atherosclerosis in chickens, Drs. T. Nishida, F. Takenaka, and F. A. Kummerow fed diets constant in their quantity of fat (corn oil) and cholesterol, but containing protein at 15, 20, 30, and 35 percent, to groups of chicks. After four weeks on these diets, they examined the blood lipids of the groups and the amount of atherosclerosis in their aortas.

The workers found that the blood cholesterol levels and atherosclerosis were significantly less at the higher levels of protein intake. "The birds fed fresh corn oil showed a decrease in the incidence of atheromatous lesions with an increasing protein level", they report in the journal Circulation Research.

When corn oil which had been subjected to 400 degrees of heat for 24 hours was substituted for the fresh corn oil in the diets, the blood cholesterol and lipids were actually lower, even when the chicks ate more cholesterol, but a more severe atherosclerosis resulted from the heated oil. "Birds fed heated oil and protein at a high level had at least as severe a degree of atherosclerosis as those fed fresh fat at a low level of protein", the investigators state.

"The present results may help to extend basic information on atherosclerosis in two directions", they explain. "One, they indicate that dietary stress factors other than a high fat, high cholesterol diet may be involved in the inducement of experimental atherosclerosis;

and two, that high serum cholesterol and high beta lipoprotein values do not serve as an accurate index for atherosclerosis."

ESTROGEN TREATMENT AFTER MENOPAUSE MAY CONTROL HIGH BLOOD LIPIDS

Elevations in the serum lipids of 35 hysterectomized women with histories of coronary disease (angina or heart attack) were lowered to levels comparable to those of healthy young women after three months of treatment with high doses of estrogens.

This finding is reported in Annals of Internal Medicine by Drs. R. W. Robinson, W. D. Cohen, and N. Higano of the Memorial Hospital, Worcester, Massachusetts, who are studying the effects of long-term estrogen therapy on the serum lipids of postmenopausal women. The research has been aided by an NHI grant.

The relative immunity of young women to coronary atherosclerosis declines after menopause. Secretion of estrogens also declines. Coincidental with this decline in ovarian hormones, there is an increase in the serum cholesterol, in the ratio of cholesterol to phospholipids, and in the amount of cholesterol in beta, as opposed to alpha, lipoproteins.

Administration of estrogens has been shown in a number of studies to revert such blood lipid patterns toward normal both in men and animals. Yet, the Worcester investigators point out, no previous studies of the effects of estrogen administration on serum lipids in women have been published since a few short-term trials in 1949.

The high dosage schedule effective in the Worcester coronary patients was a daily 5 or 10 milligram dose of oral mixed conjugated estrogens (Premarin). This dose level has previously been shown to produce similar changes in men.

In the Worcester patients this high dose level was limited to women whose uteri had been removed for gynecological reasons, because the estrogens tended to cause disturbing side effects, particularly uterine bleeding. However, in a group of 59 healthy postmenopausal women, it was found that high blood lipids could often be controlled on much lower dosage schedules with less trouble from side effects. Additional investigation, now in progress, is required to determine whether a dose optimal for lowering serum lipids will cause disturbing uterine bleeding.

Information on the longevity of estrogen-treated compared with untreated postmenopausal women will be available eventually from their continuing studies, the investigators state.

ANGINA PECTORIS RELIEVED BY STRIPPING PLUGGED-UP LINING FROM ARTERIES

Successful treatment of patients with angina pectoris by surgically removing the obstructing cores of atherosclerotic debris from their coronary arteries is reported by NHI research grantees at the University of California Medical Center in Los Angeles.

The investigators, Drs. William P. Longmire, Jack A. Cannon, and Albert A. Kattus, reporting in the New England Journal of Medicine, express the view that the most effective means of increasing the blood flow to the heart muscle of patients with angina pectoris may be a direct surgical attack on the atherosclerotic core which is blocking the coronary blood flow. On the basis of experimental and pathological, as well as logical, evidence supporting this view, they stripped the thickened inner lining, or intima, from segments of the coronary vessels of five patients whose angina pain was so severe they had been incapable of gainful employment.

In every case, the investigators report, it was possible to locate and remove occluding cores from one or more of the main coronary vessels during the operation, and thus re-establish the pulsations of blood flow through the stilled and hardened vessels. Two of the five patients were completely relieved of their crippling pain. In two of the others, the duration and severity of the pain was greatly reduced. The heart of the fifth patient stopped during the operation after obstructions had been successfully removed from two coronary vessels.

In their direct vision operations, the Los Angeles workers exposed the main coronary vessels by freeing them from their beds of fat. The diseased segments could then be identified, chiefly by touch, for they did not pulsate and the plugged portions were firm and rigid. Any possibility of their being open was ruled out by clamping off the suspect vessel for a few minutes, while closely watching the electrocardiogram and the color of the heart muscle. Clamping produced no darkening of the heart muscle or changes in the electrocardiogram, if the vessel was occluded.

Removal of the occluding core was begun by making a small lengthwise cut in the blocked-up segment and inserting a tiny blade to begin freeing the thickened intimal lining from its attachment to the medial layer of the vessel. A tiny steel loop on a shaft was then slipped over the freed end of the rigid intimal layer and worked along this core inside the artery to strip it loose in both directions from the place of the incision, so that the core over the entire length of the occluded area could be withdrawn through the incision. After the core was removed, an observable flow of blood occurred within the vessel, which began to pulsate after the incision was sewed.

"These results indicate that it is technically feasible by this procedure to re-establish blood flow in previously obstructed major coronary arteries," the investigators state. "Experience with additional cases and longer periods of observation will be necessary to make final assessment of the value of the procedure."

INHIBITOR OF CHOLESTEROL SYNTHESIS ALSO INHIBITS ADRENAL FUNCTION

Animal studies at the Heart Institute have shown that delta-4-cholestenone, a synthetic compound that suppresses cholesterol manufacture in the body, also suppresses the secretion of vital steroid hormones by the adrenal glands.

Drs. Daniel Steinberg and Donald S. Fredrickson of NHI's Laboratory of Cellular Physiology and Metabolism noted that rats receiving delta-4-cholestenone developed a remarkable enlargement of the adrenal gland, as much as 7 times normal. Delta-4-cholestenone had been shown to suppress cholesterol biosynthesis almost completely and in order to explain the observed effects on a single basis, the scientists hypothesized that the inhibition of cholesterol synthesis was leading to inhibition of adrenal steroid synthesis. This would release the pituitary gland from the control normally exercised over it by the outpouring of adrenal steroids (a negative feed-back mechanism) and thus lead to the observed hypertrophy.

It has now been shown by direct measurements of the rate of adrenal steroid output in the adrenal vein blood of the rat that delta-4-cholestenone does in fact inhibit adrenal function. Studies by Drs. Fredrickson, Ralph E. Peterson, and Steinberg, published in Science, show that the output of corticosterone, the major adrenal steroid in the rat, is decreased by well over 80% in animals receiving delta-4-cholestenone. These results are of particular interest for the light they may shed on the physiologic role of the cholesterol molecule.

Because of the close structural similarity between cholesterol and the adrenal steroids, a functional relationship has long been suspected. Over the past 10 years it has been shown by the use of isotopes that a large number of the adrenal steroids can be formed from cholesterol. However, other evidence indicated that the steroids could also be synthesized without the necessary participation of cholesterol, leaving uncertain the relative importance of cholesterol as an intermediate. These recent studies at the National Heart Institute support the view that cholesterol is an important precursor for the adrenal steroids and that when cholesterol synthesis is impaired beyond a certain point there is a consequent inhibition of adrenal steroid production.

These findings also indicate that compounds designed to lower blood

cholesterol by inhibiting synthesis, now an important area of research in the problem of atherosclerosis, will have to be carefully screened for possible deleterious effects on adrenal function.

UNSATURATED VEGETABLE OIL ADDED TO USUAL DIET FAILS TO LOWER CHOLESTEROL

Safflower oil, the most unsaturated vegetable oil available today, failed to lower the blood cholesterol when added as a supplement to the regular diets of 24 normal young men.

This finding is reported by Drs. Richard Perkins, Irving S. Wright, and Barbara W. Gatje of the Department of Medicine, New York Hospital, Cornell Medical College. "Our results", the NHI grant-aided investigators emphasize in the Journal of the American Medical Association, "are not to be construed as denying the possible effectiveness of safflower oil as a supplement to diets which have been markedly reduced in their content of animal fat. It seems definitely established", they point out, "that the careful substitution of unsaturated vegetable oils for saturated animal fats in human diets will produce a sustained reduction in total serum cholesterol level. Several unsaturated vegetable oil emulsions are now available. The temptation on all sides has been to assume that they would effectively reduce the serum cholesterol level, when given as dietary supplements, without much change in the patient's regular diet."

"But despite the widespread manufacture and prescription of safflower oil emulsions and despite the fact that large numbers of patients are taking these supplements to their normal diets in hopes of reducing their serum cholesterol levels, very little experimental evaluation of their value as such has been published."

Twenty-four young medical students--men aged 24 to 26--cooperated as subjects for the study. The habitual dietary pattern of each was determined by questionnaire, and each was required to adhere to his regular eating pattern throughout the 18 weeks of the study. After a five week control period, the subjects were given, in sequence for six week periods, a fat free carbohydrate placebo to imitate the oil, and a daily supplement of 75 cubic centimeters, or about two and one-third ounces, of 65% safflower oil.

During both the placebo and the safflower periods, the serum cholesterol actually rose slightly in most of the students. The investigators speculate that this increase may have been due to seasonal influences (the study lasted from late summer to early winter), or the additional nourishment from the oil and the carbohydrate placebo. The subjects reported weight gains averaging four and one-half pounds.

SYNTHETIC ESTROGEN THERAPY NOT UNDULY FEMINIZING, CLINICAL TRIALS SHOW

High beta lipoprotein levels in the blood of thirteen of twenty male and female coronary patients were shifted to normal (to the level of young, healthy women) during 2 to 15 months of daily treatment with a synthetic estrogen, SC 6924 (Manvene), it was found in an NHI grant-supported study at the Johns Hopkins University School of Medicine. The investigators, Drs. F.W. Davis, Jr., W.R. Scarborough, R.E. Mason, M.L. Singewald and B.M. Baker, Jr. have reported their results in The American Journal of the Medical Sciences.

Several of the more conventional estrogens also had this presumably desirable effect, but all of the men treated with conventional estrogens suffered the feminization which in the past has generally deterred the use of such female sex hormones against high blood lipids and atherosclerosis in men.

Only two of twenty SC 6924-treated men suffered the impotence and complete loss of libido usually resulting with conventional estrogens. Breast changes were noted in fifteen of twenty-six patients treated but were not as objectionable as when conventional estrogens were used, according to the investigators.

"From these relatively short-term observations, it would seem that the possibility of extended therapy with Manvene in quantity sufficient to favorably influence serum lipids exists, with prohibitive feminization occurring in far fewer patients than appears during ordinary estrogen treatment," the investigators report. They also point out that their results are preliminary, and strongly caution that this form of treatment is still experimental and should not be indiscriminately applied in the treatment of coronary atherosclerosis until more data are available.

NEW PROTHROMBIN-DEPRESSING ANTICOAGULANT, ANISINDIONE, FOUND UNUSUALLY PROMISING

Investigators of New York Medical College report that a new synthetic anticoagulant, anisindione, is unusually free from toxic side effects and relatively uniform in its anti-clotting action in different individuals over long periods of time. The investigators, Drs. Kurt Lange, Eli Perchuk, Murray Mahl, Josef Enzinger, and George Mouratoff, also report that it is orally effective in a single dose given every third day. Dr. Mahl is a Research Fellow of the Heart Institute.

Anisindione is a new member of the "indandione group" of synthetic anticoagulants, known from the work of Dr. Herman Kabat and his coworkers in the Division of Chemotherapy of NIH in 1944. Indandione anticoagulants generally inhibit the capacity of the liver to produce

prothrombin, from which the constituents of blood clots are formed. In this respect the indandiones resemble the familiar courmarin types, but differ from the body's own anticoagulant heparin, which acts more directly on the clotting mechanism.

In their report in the American Heart Journal, the New York workers emphasize the disadvantages of conventional anticoagulants in presenting their findings concerning anisindione. They point to the fact that heparin, an expensive drug, is ineffective orally and hence cannot be used for long term (maintenance) therapy. And they underscore the irregularity of response to the widely used coumarins, which makes dosage regulation of these drugs difficult. "Therefore," they explain, "even under the most careful control, hemorrhagic episodes cannot be excluded". Other anticoagulants of the indandione type also have disadvantages of variability in individual sensitivity as well as toxic side effects such as hypersensitivity reactions.

In animal experiments, it was found that anisindione displayed unusual freedom from toxic side effects on the liver and blood, and seemed to promise fairly uniform and consistent dosage schedules. So the New York workers tried the drug on 52 patients with various cardiovascular diseases, the clinical studies lasting a total of 1,199 patient days.

In these clinical studies, the drug was found to depress prothrombin to a therapeutically effective anti-clotting level within 36 to 72 hours; this level was maintained by a single dose every third day. The workers report that the individual response to a given dose is unusually uniform, that the effect can be quickly interrupted by a vitamin K preparation, and that the anti-clotting level can be restored promptly by remedication. "Side reactions", they emphasize, "were noticeably absent in this group."

PROLONGED REST UNNECESSARY IN "MILD" HEART ATTACKS, STUDIES INDICATE

Of the thousands of people who suffer heart attacks (myocardial infarction) each year, at least 15 percent have "mild" attacks and can safely be permitted to increase their activity gradually after two weeks of rest and observation, studies by Drs. Myron Prinzmetal, Rexford Kennamer, Stanley M. Weiner, and Mannish C. Bhuyan of the Cedars of Lebanon Hospital and the University of California, Los Angeles, indicate. Traditional methods of treatment call for prolonged rest, regardless of the severity of the attack, ranging from 4 to 8 weeks and averaging 6 weeks in length.

Reporting in the American Journal of Cardiology, the scientists present evidence from clinical studies and animal experiments in support of their new method of management. Their work was aided by a National Heart Institute grant.

Modern diagnostic techniques enabled the investigators to formulate detailed criteria for detecting "mild" cases and differentiating them from the "moderate" and "severe" cases of myocardial infarction.

In the typical "mild" case, the patient is young or middle-aged, and has not had previous heart disease, hypertension, diabetes or other chronic illness. The pain of the attack is of short duration and does not recur. Fever is slight or absent, the serum transaminane level only moderately elevated, white blood cell increase mild, and the red cell sedimentation rate only slightly increased. Shock, heart enlargement, gallop rhythm or other significant abnormal rhythms, and signs of heart failure do not occur. The patient looks and feels well a day or two after the attack.

Using these criteria, the investigators classified 200 consecutive heart attack patients admitted to Cedars of Lebanon Hospital. They found 15 percent were in the "mild" category. They believe this figure to be low as an index of the "mild" cases occurring generally, since some patients do not receive hospitalization and are treated at home, and other cases may remain undiagnosed.

The investigators also reviewed the clinical records of 400 patients who died of myocardial infarction at the hospital during a period of six and one-half years. They found that not even one of these patients could be classified as "mild", indicating the low mortality rate of patients who can be so classified by their criteria.

The threat of ballooning or rupture of an infarcted heart before a firm scar can be formed is often given as the main reason for keeping all heart attack patients in enforced rest for long periods. Dr. Prinzmetal and his co-workers point out that "in mild cases, the risk of rupture after two weeks of rest is nil" and that the great majority of cases developing these complications also have hypertension, a condition which excludes them from the "mild" category. They also demonstrated in dog experiments that two weeks is sufficient time for the formation of a strong scar after small infarcts. They found it "virtually impossible" to rupture such experimental infarcts at fluid pressures as great as those found in human hearts.

The investigators have observed or treated 54 patients with "mild" infarctions whose length of treatment was less than the number of weeks conventionally prescribed. Twenty-two have been followed for more than a year. Some have been followed for as long as 10 years. No untoward effects were seen in any of the patients.

"In view of the very low mortality and paucity of complications in 'mild' cases," conclude the Los Angeles workers, "it seems neither necessary nor desirable to subject such patients to the same rigorous and prolonged treatment required for more severe cases. Furthermore, early activation of these patients results in distinct physical, psychologic and financial benefits." The investigators emphasize, however, that classification should be made only after two weeks of conventional treatment and should be followed by careful weekly re-evaluations of each case for about one month.

HIGH BLOOD PRESSURE

POTENT MONOAMINE OXIDASE INHIBITOR FOUND TO BE ANTIHYPERTENSIVE AGENT

A new drug, 1-phenyl-2-hydrazinopropane, also known as JB 516, produces marked and sustained lowering of blood pressure in patients with hypertension, it has been found by NHI scientists Drs. Louis Gillespie, Luther L. Terry, and Albert Sjoerdsma. A summary of their findings, presented at the 1958 Scientific Sessions of the American Heart Association, has been published in the journal Circulation.

The investigators report that the antihypertensive effects of JB 516 appear to resemble those of certain pressure-lowering drugs (ganglionic blocking agents) currently in use. Unlike the latter, however, JB 516's side effects do not include dry mouth, sexual impotence and constipation, according to the investigators.

In some patients given high doses of this new drug, the investigators noted a unique side effect--temporary development of red-green color blindness. This side effect has proven to be reversible upon withdrawal of the drug.

Synthesized by a pharmaceutical concern, JB 516 is one of a series of monoamine oxidase inhibitors which have been under investigation by Heart Institute scientists seeking an improved method of treating high blood pressure. These inhibitors are drugs which counteract the enzyme, monoamine oxidase (MAO) which usually destroys certain amines in the body, such as serotonin and norepinephrine. These amines influence blood pressure, heart rate, emotions and other involuntary functions in a way not yet understood. Of the MAO inhibitors studied at the Heart Institute to date, JB 516 is by far the most potent, according to Drs. Gillespie, Terry and Sjoerdsma.

Following NHI laboratory investigations of the compound in experimental animals, the studies were carried out in 21 patients afflicted with severe essential hypertension. Eight of 9 hospitalized patients, given single daily doses for short term periods of twelve to twenty-five days, showed effective response to the drug. Eleven of 12 outpatients, treated for periods of two to five months, responded with significant and sustained lowering of standing blood pressure. The hypertension of one patient, previously requiring four separate drugs for proper control, was found after seven weeks' treatment to be adequately managed with JB 516 alone.

The investigators point out that it is too early to determine the extent to which JB 516 will be of lasting value in the treatment of

hypertension and that expanded clinical tests must now be made. Their findings indicate, however, that monoamine oxidase inhibition may represent a promising new approach to more successful treatment of hypertension, as well as opening the door to increased understanding of the manner in which the nervous system chemically influences blood pressure.

LOWERING BLOOD PRESSURE MAY REDUCE CAPACITY OF KIDNEYS TO EXCRETE SODIUM

The increased capacity of hypertensive persons to excrete sodium is often reduced by effective antihypertensive therapy, Boston investigators report. The findings suggest that the blood pressure itself may operate to control sodium excretion by the kidneys.

Drs. William Hollander and Walter Judson of the Massachusetts Memorial Hospitals and Boston University School of Medicine report that effective antihypertensive therapy, either by drugs or by surgery, often reduces the heightened sodium excreting capacity of hypertensives toward normal.

The findings were made, with the aid of NHI grant support, in detailed renal function studies of patients with essential hypertension. A full report of the studies has been published in the journal Circulation.

The fall seen in sodium excreting capacity appeared from the renal function tests to be due to change in the activity of the kidney tubules, not from any change in body fluid and salt balance.

The findings thus suggest to the Boston workers that the arterial blood pressure itself plays a major role in regulating sodium excretion by the kidneys. The findings also suggest that some of the disturbances in fluid and salt metabolism seen in persons with hypertension may be the result, rather than the cause, of their high blood pressure.

NERVE STRUCTURES THAT HELP REGULATE BLOOD PRESSURE MAY EXIST IN ABDOMEN

The existence of pressure-sensitive nerve structures (pressoreceptors) in the area of the pancreas in cats, which contribute substantially to the normal regulation of blood pressure, is indicated by experiments by Drs. Stanley J. Sarnoff and Samuel I. Yamada of the National Heart Institute's Laboratory of Cardiovascular Physiology. The finding has been reported in Federation Proceedings.

Only two such blood pressure-regulating nerve pressure sensors have previously been known. One, the carotid sinus, is a network of nerve fibers originating in the wall of the carotid artery, just above the point in the neck where it divides to supply different parts of the head. The other is a similar network in the arch of the aorta, where this great artery curves from the top of the heart to supply all body tissues.

Responding to the stretching caused by the thrust of blood within their respective arteries, these receptors reflexly stimulate impulses that regulate blood pressure by altering the caliber of arterioles and the rate of the heart. Thus, increased pressure within these presso-receptor sites stimulates nerve impulses that dilate arteries generally, slow the heart, and lower the blood pressure. Conversely, lowering of pressure in these sites results in an increase in arterial blood pressure.

Such an increase in blood pressure was produced in cats by Drs. Sarnoff and Yamada by temporarily lowering blood pressure in various abdominal arteries. Lowering pressure in the pancreatic arteries produced an especially striking reflex increase in blood pressure--often greater than the increase that results when pressure in the carotid sinuses is similarly reduced.

This suggests to the NHI investigators the existence of a third site of pressoreceptors--one in the abdomen that reports to blood pressure-regulating centers the state of the circulation in organs of that region.

Drs. Sarnoff and Yamada believe that Pacinian corpuscles in the abdomen may function in this capacity. These pinhead-sized nerve endings (named after the 19th century Italian anatomist, Filippo Pacini) are enveloped in many thin onion-like layers of connective tissue. They are known to mediate deep pressure sensations from various locations in skin, connective tissues, tendons, and joints. In the abdomen, where they are found adjacent to the arteries that supply various organs--especially the pancreas--their function has long been in doubt.

However, twenty-three years ago two physiologists named Gammon and Bronk reported from studies at the University of Pennsylvania that increasing arterial blood pressure in the cats would cause increases in the number of impulses discharged by adjacent Pacinian corpuscles. The observations made in the NHI are in accord with and give physiological significance to the observations of Gammon and Bronk.

In addition to the fundamental value of adding to knowledge of normal mechanisms for blood pressure control, these findings may help explain the bizarre variations in blood pressure that accompany the

manipulation of abdominal organs during surgery. It is also possible, the investigators point out, that the continuing investigation of these receptors will reveal ways of activating them in animals so as to produce a better type of chronic experimental hypertension than has been available through operations on the two previously known pressoreceptors.

STUDY CHALLENGES EXISTENCE OF "NEW" RENAL PRESSOR REPORTED BY RUSSIANS

A series of Russian publications over the past four years has described a substance called "renol", obtained from mammalian kidneys, which is capable of raising the blood pressure. Renol, the Russian scientist O. A. Stepun and co-workers report, is distinct from renin, another renal pressor discovered in 1898.

On the assumption that knowledge of a second pressor agent from the kidney might help clarify still unknown mechanisms underlying renal hypertension, Drs. Erwin Haas and Harry Goldblatt of the Beaumont Memorial Research Laboratories of Mt. Sinai Hospital and the Institute of Pathology, Western Reserve University, undertook a study of renol. Their findings from this NHI grant-aided study are reported in Laboratory Investigations.

The Cleveland workers carefully duplicated the methods outlined in the Russian literature for isolating renol from rabbit kidneys. The substance thus isolated was subjected to a variety of tests. The investigators found that the recorded pattern of blood pressure elevation produced in dogs by injections of the "renol" preparation was identical to that produced by purified renin. Also, antirenin, an antienzyme produced in the dog by repeated injections of renin, was found to destroy "renol" just as it does renin. Further, "renol", like renin, was also found to react with plasma angiotensinogen to produce angiotensin, the arteriolar constricting substance that actually raises the blood pressure when renin is released in the body.

"The results indicate that renol is identical with renin", Drs. Haas and Goldblatt report. They attribute the failure of the Russian workers to recognize this identity to their failure to notice the presence of interfering substances in their preparations, their use of an unsuitable assay procedure and their failure to evaluate correctly certain chemical properties of renin.

SURGERY STILL HAS MUCH TO OFFER PATIENT WITH SEVERE UNYIELDING HYPERTENSION

Either sympathectomy or adrenalectomy will help the majority of operable patients with severe hypertension who have failed to respond satisfactorily to antihypertensive drugs, investigators at the University of Pennsylvania have concluded after a three-to-seven year follow-up study in 230 surgically treated hypertensives.

Drs. W. S. Blakemore, H. A. Zintel, W. A. Jeffers, A. M. Sellers, A. I. Sutnick, and M. A. Lindauer participated in the study, which derived Heart Institute support from a research grant to Dr. Jeffers. Dr. Sutnick was a Research Fellow of the Heart Institute.

One hundred and sixteen patients at the University of Pennsylvania Hospital were treated for advanced hypertensive vascular disease by removal of all or nearly all of their adrenal gland tissue and three of the artery-constricting spinal sympathetic ganglia (a "modified Adson sympathectomy"). Another 114 patients received a more extensive sympathectomy (seven of the ganglia were removed) but no adrenalectomy. All 230 patients were followed for 3 to 7 years after treatment in a study of the comparative effects of the two kinds of surgical treatment.

Dr. Blakemore and co-workers, who report the study in the journal Surgery, found that the procedure including adrenalectomy produced superior results on blood pressure, damaged retinal blood vessels, electrocardiograms, and heart size. Both procedures benefited angina pectoris, congestive heart failure, and headache, although the effects of adrenalectomy are superior for angina and congestive failure. Operative and postoperative mortality, however, were somewhat higher for adrenalectomy. (Sixty-eight percent of the adrenalectomy patients survived during the follow-up compared with seventy-three percent of those receiving sympathectomy alone.)

The degree to which these seriously ill patients can be rehabilitated by both procedures is encouraging, Dr. Blakemore's group concludes.

"In our recent experience," they emphasize, "we have been dismayed at the increasing numbers of patients who have been referred for operation too late in the course of their disease, after unsuccessful trials with a variety of vasodepressor agents. It would seem worthy of emphasis that surgical procedures still offer additional help to a considerable number of patients with advanced hypertensive vascular disease in whom medical treatment has failed to halt the progressive pattern of the disease."

SURGERY

DETECT ABNORMAL OPENINGS IN HEART PARTITION WITH USE OF KRYPTON^{85} GAS

Research at the National Heart Institute has shown that Krypton^{85}, a radioactive form of a harmless inert gas, can be used to detect abnormal openings in the wall of muscle dividing the right and left chambers of the heart. More than 100 patients with proved forms of heart disease have now been studied, and Krypton^{85} has made possible the correct diagnosis in every instance.

Left to right "shunts" of blood which result from defects in the partitions of the heart are the commonest form of congenital heart disease. Accurate knowledge of the presence and location of these defects is essential for corrective surgery.

Details of the diagnostic technique, which was developed by Dr. Richard Sanders of the Heart Institute's Clinic of Surgery, have been reported in Proceedings of the Society for Experimental Biology and Medicine.

Experiments using a different radioactive inert gas successfully in detecting heart abnormalities are reported in the same journal. Dr. Sanders bases his choice of Krypton on its complete inertness and ready availability. In addition, 99 percent of Krypton^{85}'s radiation is nonpenetrating, minimizing the elaborate safety precautions generally necessary for the protection of laboratory workers who are repeatedly exposed to radioactivity.

Krypton^{85} can be measured instantly and accurately with an instrument similar to a Geiger counter after the gas has been introduced into one side of the heart and some of it has been recovered from the other. This makes the test simpler than any previous laboratory techniques, and the test is more accurate than the standard techniques now being used in most heart clinics.

In developing the technique, Dr. Sanders used dogs with heart defects which produced left to right shunts. He introduced radioactive Krypton into the left side of the heart by having the dogs breathe it, mixed with air, from an anesthesia bag. The radioactive gas was instantly picked up by the bloodstream from the lungs and carried to the left side of the heart, along with normally oxygenated blood destined to supply the dog's whole body.

In a perfect heart, this blood does not enter the right side of the heart until it has completed the circuit through the arteries to the body and returns through the veins into the right side of the heart ready to be sent, from the right side, to the lungs for a new oxygen supply.

In Dr. Sanders' experimental animals, the normally high pressure in the left side of the heart forced the blood not only into the artery leading to the rest of the body, but also through the holes in the partition between the left side of the heart and the right. This robbed the dog's body of part of its needed blood supply and is what happens in a human being with congenital heart defects in which the blood is shunted from left to right.

Using the technique of catheterization, with his experimental animals, Dr. Sanders threaded a length of tiny plastic hose through a neck vein feeding into the right side of the heart. Through this tube, he could draw blood directly from the right side. In a normal dog breathing radioactive Krypton, samples of blood showed very little radioactivity--the radioactive gas had been largely dissipated on the long journey of the blood around the body.

When Dr. Sanders tested blood from the hearts of the dogs with heart defects, however, he found it to be much more radioactive, which showed the presence of the defects. By "aiming" the tip of the catheter at different parts of the heart, the location of the defect can be determined.

Many heart defects involving left-to-right shunts can now be repaired by surgery, but more than 1,000 persons, most of them children, die of these defects each year in the United States, and thousands more are crippled.

COMBINATION OF SALTS FOUND SUPERIOR FOR STILLING HEART DURING SURGERY

Measuring with sensitive instruments the effects of several different groups of heart-stopping substances on the contractility and electrical activity of isolated cat hearts, Drs. D.M.H. Merritt, W.C. Sealy, W.G. Young, and J.S. Harris of Duke University found both potassium citrate and magnesium sulfate to have the desirable effect of stopping all heart action when either salt was perfused through the coronary arteries. But afterward, when attempting to restore the beat, the potassium salt required about three minutes of perfusion before normal heart action returned. The magnesium salt, on the other hand, would allow a normal beat to return almost immediately on flushing the coronaries, although the magnesium was slower to arrest the heart initially.

The investigators, whose studies were supported in part by a National Heart Institute grant, then combined the two salts in various proportions, seeking a more nearly ideal effect i.e., more immediate arrest and recovery, without complicating side effects. They report finding a combination with speed of action and of recovery greater than those

of either salt alone. Using this combination they were able to start and stop the cat heart nine times in 3½ hours without significant evidence of consequent damage. Neither of the salts was as effective alone, they report. The neostigmine was included because it was found capable of slowing the heart beat (and hence its oxygen demands) during the recovery period, without interfering with the return of the normal beat.

"During open heart surgery there is much to be gained from having a still heart", the investigators explain in presenting their findings in the A.M.A. Archives of Surgery. "The danger of air embolism is minimized; blood loss is lessened, and the surgeon has a clearly visible and motionless operative field. The presumed tolerance of the stilled heart to ischemia (oxygen deficiency) might be still another important advantage. In hypothermia, operations on the ventricles would not be feasible without a method of control of the heart action."

NEW HEART-LUNG MACHINE OXYGENATES BLOOD THROUGH POLYETHYLENE MEMBRANES

A new heart-lung machine which offers some theoretical advantages over other machines has been devised and successfully used by Drs. F. John Lewis, Riccardo Benvenuto, and Nicholas Demetrakopoulos of Northwestern University Medical School. The "lung", or oxygenator, of the machine performs in a manner similar to the living lung in that very thin layers of blood are shielded against direct contact with air by membranes through which oxygenation and carbon dioxide release occur.

The oxygenator consists of from four to sixteen (as many as are needed for the anticipated blood flow) polyethylene tubes. Each tube is stretched flat on a metal frame approximately two by four feet, is provided with lucite discs at the top and bottom for blood entry and exit, and stacked one on top of the other. Aluminum dividers placed between the polyethylene membranes have a design in rubber on one side which presses against the adjacent flattened polyethylene tube, forming baffles that channel blood flow down between the tube walls. Thus blood is distributed, not in rivulets as in other membrane-type oxygenators, but in an extremely thin film, allowing more efficient oxygenation. Oxygen flows through tubes into one side of the dividers separating the membranes and out through holes at the opposite side.

The machine has two pumps--one circulates blood through the oxygenator and the other pumps the oxygenated blood back into the subject. Close supervision of the machine during an operation is not necessary, the investigators report, because blood flow is automatically maintained through a pump control unit which responds to fluctuations

in the level of oxygenated blood in a reservoir. Since blood flowing through the machine is not excessively stirred or exposed to air, risks of air bubbles, fibrin particles, and blood protein changes have been virtually eliminated. Another advantage cited by the investigators is that since the oxygenating membranes are disposable, cleanliness presents no problem.

A detailed report on the new heart-lung machine has been published in the Quarterly Bulletin of the Northwestern Medical School. The research was supported in part by an NHI grant. In its first clinical trial following preliminary trials in dogs, the machine successfully maintained the circulation of a 44-year old, 140-pound man during an operation for aortic stenosis.

METHOD OF DETECTING MITRAL INSUFFICIENCY DEVELOPED AT THE HEART INSTITUTE

The finding in dog studies at the National Heart Institute that increased resistance to ejection of blood from the left ventricle of the heart elevates pressure in its left atrium has provided a basis for a clinically valuable method for detecting insufficiency (leakage) of the mitral valve in difficult cases.

In the dog studies, Drs. Eugene Braunwald, George H. Welch, Jr., and Andrew G. Morrow used clamps to resist the ejection of blood from the left ventricle. They found that the blood, when thus confined in the contracting ventricle, tended to seek "the back way out" through the mitral valve. If the mitral valve was incompetent, its tendency to leak was thus amplified and the blood rushing back through it into the atrium raised the pressure in this chamber much higher than when the valve did not tend to leak.

Following successful trials in dogs, the method was employed in twenty patients--seven with pure mitral insufficiency, seven with pure mitral stenosis, and six with mixed insufficiency and stenosis. Norepinephrine infusions, rather than clamps on the aorta, were used in the patients to produce transient, controllable, and safe increases in resistance to left ventricular ejection.

In the thirteen patients with insufficiency, the artery-constricting norepinephrine increased left atrial pressure to an average of 47.8% of the rise it produced in arterial pressure--in some patients it went as high as 125%. In the patients with pure stenosis the increase in atrial pressure averaged only 8.5% of the arterial pressure increase. The difference was great enough to form the basis for a method for detecting mitral insufficiency.

The new method, which has been reported in the Journal of Clinical Investigation, is expected to be most useful in the combined condition where the murmurs and blood pressure abnormalities of insufficiency are often masked by those of stenosis.

HEAT-SEALED DACRON GRAFTS FOUND ADVANTAGEOUS AS ARTERY REPLACEMENTS

Grafts of Dacron taffeta, individually fashioned at the operating table by cutting two pieces of the flat cloth to desired dimensions and heat-sealing their edges together to form a tube, give excellent results as replacements for diseased portions of blood vessels, surgeons at George Washington University have found.

The investigators, Drs. Owen Gwathney, Howard C. Pierpont, and Brian Blades, have reported in the journal Surgery on their clinical experiences with such grafts. Following experiments with animals to establish the safety of the technique, heat-sealed grafts were successfully implanted in five patients -- two with aortic aneurysm (ballooning of the blood vessel wall) and three with thrombo-occlusive disease (blocking of the artery by extensive blood clotting). In each case the lesion involved the aortic bifurcation, the place where the aorta branches into two arteries going to the legs, and thus Y-shaped grafts were required.

In forming the grafts, exact measurements were made at the operating table to determine the size needed for the patient. Two pieces of Dacron cloth were then placed between the two plates of a proper sized aluminum mandrel or template. The fabric was cut to allow 3 to 4 millimeters for heat sealing, and the sealing was done with an alcohol lamp just outside the operating room to prevent explosions.

The NHI grant-aided investigators were prompted to experiment with methods of improving and simplifying the construction of Dacron fabric grafts because of disadvantages encountered in their previous clinical experience with graft surgery. For example, they found that with Vinyon-N and nylon seamed grafts, machine-sewed to predetermined sizes, no satisfactory adjustments could be made in length or width. Cuffing to decrease length reduced the opening, caused wrinkling, and the resulting thickness created difficulties in stitching the graft to the host vessel. With braided seamless tubes, much thicker than those made from sheets of cloth, alarming blood loss through the fabric was also frequently encountered.

Use of the heat-sealed, tailored grafts obviate these difficulties. The investigators report that sweating or hemorrhage of these grafts was minimal and there was no appreciable bleeding at the suture line. They stress, however, that the principal advantage of the heat-sealed Dacron graft is that it can be fashioned at the operating table to meet the needs of each individual case.

CO_2 GAS USED TO PREVENT AIR EMBOLIZATION DURING HEART SURGERY

The deadly surgical accident of trapped air bubbles blocking the coronary blood supply to the heart muscle may become increasingly common with the increased use of open heart surgery.

To reduce this possibility, Philadelphia surgeons H. T. Nichols, D. P. Morse, and T. Hirose flood the empty and exposed chambers with carbon dioxide gas at the end of an open heart operation, just before the blood is allowed to well up into the heart from the clamped-off veins. Thus, they report in the journal Surgery, any small but dangerous bubbles of air which might be caught in the heart's inner crevices and irregularities are replaced by a gas which is so rapidly absorbed into the blood that it is clinically harmless.

They report using this method without ill effects in 22 consecutive open heart operations at Hahnemann Medical College and Hospital of Philadelphia. The work was done while Dr. Morse was a trainee of the National Heart Institute.

These investigators feel that the risk of accidentally trapping air in the heart, with subsequent passage into the arteries that supply the body organs and tissues, including the heart muscle, is inherent in every case in which the arteries or the left chambers of the heart are opened, either directly or through a defect between the two sides of the heart.

Traveling with the blood through the tapering lumen of an artery, such air bubbles may finally lodge in a coronary or cerebral vessel. Being relatively insoluble, air will block the blood flow through such arteries long enough so that vital heart or brain tissues are destroyed.

Carbon dioxide gas is about twenty times as soluble as air and its bubbles are entirely taken up by the blood within a much shorter time before any such damage can occur, the surgeons explain.

BACKFLOW OF BLOOD THROUGH AORTIC VALVE DETECTED AND ESTIMATED BY NEW METHOD

An indicator dye technique developed at the Heart Institute permits not only the detection of "leaky" aortic valves, but also the estimation of the volume of blood leaking back through the incompetent valve.

The value and safety of the new method, announced in the journal Circulation by Drs. Eugene Braunwald and Andrew G. Morrow, have already been demonstrated in more than 40 patients in the Heart Institute's Clinic of Surgery. The method was found particularly

useful in detecting and assessing aortic regurgitation (blood backflow) in patients in whom this condition co-exists with aortic stenosis (failure of the valve to open fully). Detecting significant regurgitation in such patients may be of critical importance in indicating the choice of corrective surgery. The new method has also provided an index of the effect of surgery on the diseased valve.

To reveal the regurgitation, a cardiac catheter is threaded up into the aorta through a thin walled needle inserted into the femoral artery in the thigh. The tip of the catheter is fluoroscopically positioned distal to, or below, the heart and its diseased valve and downstream from the arch of the aorta with its branches to the head and arms. Dye is then injected through the catheter. Regurgitation is revealed if the dye subsequently appears in the patient's right earlobe, for to reach the innominate artery to the earlobe, the dye would have to flow "upstream" into the aortic arch where this artery originates.

In all of the group of seven patients without aortic insufficiency, dye injected below the aortic arch could not be detected flushing through the earlobe. In all 10 of the group known to have aortic insufficiency, the indicator dye did regurgitate back into the aortic arch and thus could be detected in the earlobe.

To estimate the volume of regurgitation, dye was injected at 2 centimeter intervals while the catheter was withdrawn through the descending aorta. An X-ray picture was taken of the catheter tip in its position at the lowest point from which injected dye could be detected in the ear. The volume of regurgitated blood was represented by the volume of blood in the aorta between the catheter tip and the innominate artery.

NHI TECHNIQUE EVALUATES RESULTS OF VALVE SURGERY AT TIME OF OPERATION

A technique for evaluating the effects of heart-valve surgery while an operation is in progress has been developed by Drs. Herbert Tanenbaum, Eugene Braunwald, and Andrew G. Morrow of the National Heart Institute's Clinic of Surgery. With this technique, direct measurements are made of the heart's output of blood and of differences in pressure on each side of abnormally narrowed, or "stenosed," valve openings just before and after these openings are surgically enlarged.

The new technique allows a more precise and immediate evaluation of the results of heart valve surgery than has previously been possible with conventional techniques, by which pressures and output of blood are measured before and after an operation but not during it. By making these measurements while an operation is going on, the new

technique tends to eliminate the influence of all variables except the critical one--the size of the valve opening. It gives the surgeon a better idea of how much of the valve's function has been restored and lets him know whether to enlarge the valve opening still further during the same operation.

The new method, which has been reported in The New England Journal of Medicine, is applicable in operations for stenosis of any of the four valves of the heart. Its value was demonstrated in studies of twenty-four patients undergoing valve surgery at the Heart Institute (18 with mitral, four with aortic, and one each with pulmonic and tricuspid valve stenosis).

After exposing the heart to view, the Heart Institute team measures pressures simultaneously in the chambers immediately upstream and downstream from the diseased valve by puncturing these chambers with fine (20-gauge) hypodermic needles. The needles are attached directly to two hand-held pressure transducers, which change the pressure pulses coming through the needles into electrical pulsations. The pulsations from both transducers are projected as two parallel tracings moving across the screen of a cathode ray oscilloscope. The difference between these synchronized pressure tracings reflects the pressure difference, or "gradient," across the diseased valve. The effect of the surgery in relieving the abnormal pressure gradient is apparent when these measurements are repeated immediately after the surgeon opens the heart and inserts his finger (or an instrument) into the valve opening to enlarge it.

To determine the heart's output of blood just before and after this valve manipulation, the Heart Institute team injects measured amounts of a special blue dye directly into either the pulmonary artery or the left atrium, which carry the blood from the lungs to the left ventricle to be pumped to all body tissues. As it circulates through the heart and out into the arteries, the dye-containing blood is drawn at a constant rate from an arm artery through a densitometer (a photoelectric device that detects the dye concentration). The concentration of the dye and its transit time through the circulation are also projected visually as a "dye dilution curve." The characteristics of this curving line indicate the output of blood from the heart.

Stenosis of heart valve openings is a common form of heart disease which may be inborn. More often, however, it is due to repeated episodes of rheumatic fever. This disease attacks the delicate mobile "leaflets" or "lips" of the valve, which normally close together tightly and open widely at each heartbeat to regulate flow in and out of the chambers. The adhesive inflammations of rheumatic fever may progressively fuse the lips together. This narrows the opening against the passage of blood, creating abnormal pressure gradients between the adjacent chambers, and overworking the heart.

The operation most commonly used to free these stenosed valve components and relieve the abnormal pressure gradients is called "commissurotomy," for the commissures or lines where the lips of the valves come together. In the past, accurate evaluation of the results of commissurotomy has been dependent on measuring pressure gradients through a flexible plastic catheter tube threaded into the heart from outside the body during the periods before and after the operation. During these comparatively lengthy and eventful intervening periods, factors other than surgery on the valve--such as variations in the patient's medical therapy, stress and rest experiences--can alter the pressure and flow patterns within the heart and thus mar the precision of the evaluation data.

LEAKING AORTIC VALVE MAY BE IMPROVED BY EXCISING ONE OF ITS THREE CUSPS

Investigators at Mount Sinai Hospital in Minneapolis and the Department of Surgery of the University of Minnesota are successfully converting the tricuspid aortic valve of dogs into a bicuspid valve. The principle and method of the operation developed may have clinical application in selected cases of human aortic insufficiency.

In certain forms of aortic insufficiency, the Minnesota scientists explain, the "leaky" valve condition is characterized by a dilation of the valve ring as well as a loss of substance in its mobile components, or cusps. By removing a triangular wedge of tissue at the base of the aorta along with the non-coronary aortic cusp, the circumference of the aorta can be reduced one third at the valve ring when the cut is sewn together. The remaining two coronary cusps close together more tightly within the smaller ring.

The investigators, Drs. Joseph Garamella, James Anderson, and Ruben Oropeza, have published the findings of their NHI grant-aided research in the journal Surgery, Gynecology, and Obstetrics. Twenty consecutive animal experiments are reported, as well as a functional evaluation of the "bicuspid" valve created in the animals. Survival improved as the investigations progressed, and seven of the last nine treated animals survived.

LEFT CORONARY ARISING FROM PULMONARY ARTERY SHOULD BE TIED OFF EARLY IN LIFE

The rare and usually fatal congenital anomaly of a left coronary artery arising from the pulmonary artery has been surgically treated in the past by constricting the pulmonary artery above the origin of the coronary so as to raise the pressure in the abnormal vessel. The assumption behind this therapy is that the flow of blood in such

cases is from the pulmonary artery into the myocardium (heart muscle) and that heart damage results because the pulmonary artery pressure is too low for adequate blood flow through the abnormal coronary artery to the heart muscle.

Drs. Robert B. Case, Andrew G. Morrow, Wendell Stainsby, and John O. Nestor have published evidence in the journal Circulation that the flow is in the other direction--that the heart damage results from loss of myocardial blood supply from the right coronary across abnormal communications with the low-pressure left, and into the pulmonary artery.

This evidence was obtained in part from studies on the heart of a patient who had died as a result of this condition. Dye injected into the aorta of this heart was found to appear immediately and without resistance in the pulmonary artery, revealing the free communications and indicating that the line of least resistance was in this direction.

Additional evidence from angiocardiographic studies in living patients and from reports from other investigators bear out the observations of the NHI investigators.

Surgically tying-off the anomalous coronary at the point of its origin converts the abnormality into that of a single coronary supplying both ventricles--a condition usually compatible with a normal life expectancy, the investigators explain. However, they point out, to be of any value such a procedure would have to be undertaken very early in life because of the severity of the heart damage that soon results from this congenital anomaly.

NEW KNOWLEDGE AND METHODS

NEW NOREPINEPHRINE ASSAY FRUITFULLY APPLIED IN BIOCHEMICAL STUDIES

Early in 1958, Dr. Parkhurst A. Shore and Mrs. Jacqueline S. Olin of the National Heart Institute reported in the Journal of Pharmacology and Experimental Therapeutics the development of a simple method for extracting norepinephrine from tissues and converting it into a fluorescent derivative which could be precisely measured in a spectrofluorometer.

This assay method has since been applied extensively by NHI biochemists to detect changes in tissue norepinephrine in response to various drugs. From these studies have resulted many of the findings announced during 1958 concerning the pharmacology of reserpine and related tranquilizers, and of iproniazid, 1-phenyl-2-hydrazinopropane, and other agents which inhibit monoamine oxidase.

The discovery that brain norepinephrine can be liberated by not only the Rauwolfia type tranquilizer, but also by a new synthetic tranquilizer, tetrabenazine, was one such finding. This and related discoveries have strengthened the concept that brain amines are involved in the central actions of drugs.

The recent finding that brain norepinephrine levels in certain species are increased by treatment with monoamine oxidase inhibitors was also made by the application of the new norepinephrine assay method. Such findings suggest that an increase in brain norepinephrine may be at least partially responsible for the central stimulating effects of the monoamine oxidase inhibitors--the effects which have been so enthusiastically acclaimed by many psychiatrists. The findings also admit the possibility that norepinephrine accumulation in the brain may produce some of the other clinically interesting effects of the monoamine oxidase inhibitors, such as their ability to lower high blood pressure, and block convulsions induced by electric shock and metrazol.

Research application of the new assay method for norepinephrine is expected to increase with the current expansion of interest in norepinephrine and the pharmacology of compounds which seem to involve norepinephrine in their mechanisms of action.

ALPHA LIPOPROTEIN MAY PLAY SPECIFIC ROLE IN DIETARY FAT TRANSPORT

National Heart Institute scientists have proposed, for the first time, a distinct role in dietary fat transport for alpha lipoprotein, one of the three major classes of fat-carrying molecules that circulate in the body.

Evidence presented in Federation Proceedings, by Drs. Martin Rodbell and Donald S. Fredrickson of NHI's Laboratory of Cellular Physiology and Metabolism, indicates that alpha lipoprotein may function specifically to transport newly absorbed fats from the intestine through the lymph to the blood stream.

The findings also revealed the existence and possible function of a new lipoprotein which is made in the intestine and forms a part of the chylomicrons, the large fat particles that appear in the circulation following a fatty meal.

Three major classes of lipoproteins--the chylomicrons, the alpha lipoproteins, and the beta lipoproteins--have been known for a number of years to scientists concerned with fat metabolism. By means of combination with such water-soluble protein "vehicles", the insoluble fats are transported, from digestion in the gut to metabolism in the tissues, through the various watery fluids of the body.

Past studies have shown that the chylomicrons are formed as digested fat is absorbed through the intestinal wall into the lymph vessels that drain into the blood stream. From their origin, the route they travel to the blood, and the fact that they appear only briefly in the circulation following a fatty meal, they have been considered the primary means of transporting fat from digested food to the tissues.

The transport role of the alpha lipoprotein has been more puzzling to scientists, for it has been observed to circulate continuously in the blood, where it has appeared to carry very little fat, compared with the chylomicrons. But radioactive tracer studies at NHI have now revealed alpha lipoprotein in another form--combined with the chylomicrons in the intestinal lymph vessels, where it apparently functions in transporting the fat to the blood when fat is eaten.

The previously unknown intestinal lipoprotein, made in the intestine, is incorporated into the chylomicrons there, along with the digested fat. This lipoprotein appears from the tracer studies to remain with the chylomicron throughout its journey through the circulation, for when the chylomicron disappears from the blood (presumably through capillary walls to the tissues) the intestinal lipoprotein seems to disappear along with it. This lipoprotein, the scientists speculate, may aid in transport across capillary walls.

Thus, scientists can now explain the possible transport functions of two of the three major classes of fatty particles in the circulation. The third large category, that of the beta lipoproteins,

is also believed to play some transport role, for beta lipoproteins are known to increase in the blood of persons on a diet rich in animal fats. Although the new NHI findings suggest no transport role for the beta lipoproteins, they do indicate that this class is distinct from the chylomicrons, as it does not appear to be involved in the transport of digested fats from the intestine.

DIGITAL BIOPSY TECHNIQUE VALUABLE FOR STUDY OF PERIPHERAL CIRCULATION

Drs. John Phillips and George E. Burch of Tulane University have reported a safe, simple, and relatively painless technique for obtaining samples of vascular tissue from fingers and toes. Describing their method in The American Journal of the Medical Sciences, they point out its usefulness for studies of the peripheral circulation in both vascular diseases and normal states. The research was aided by a National Heart Institute grant.

A tiny (3mm) cylinder of tissue is cut in the side of a locally anesthetized digit with a sharp skin punch, then held gently at its edge by small forceps, and clipped at its base with sharp, pointed scissors. Lifted from the digit, it is quickly placed in a fixing solution. The investigators report that bleeding is easily controlled and no sutures are necessary. The technique has been used without difficulty in over 150 patients.

The investigators state that digital biopsy offers advantages for study of the peripheral circulation because the digits contain one of the most sensitive vascular beds of any in the body, and more arteriovenous communication than any other site. Blood vessel manifestations of generalized diseases occur early in the digits, the scientists point out, and biopsy studies of these areas may improve understanding of widespread vascular changes.

STUDIES SUGGEST INTESTINE IS "OILY BARRIER" TO ABSORPTION OF DRUGS

Studies at the National Heart Institute on the absorption, distribution, and excretion of drugs increasingly support the concept that cell membranes are lipoid, or fat-like, barriers which drugs penetrate by virtue of their fat solubility. Recent animal studies on drug absorption from the digestive tract strengthen this thesis.

Heart Institute scientists have found that the passage of drugs across the small and large intestine of the rat is determined by how

strongly acid or basic they are (the extent to which drugs in solution are dissociated into negatively or positively charged molecules). Thus very strongly acid or basic drugs (strongly dissociated electrolytes) are hardly absorbed at all, but drugs which are only weakly acid or basic (relatively undissociated electrolytes) are readily absorbed into the body.

These findings were disclosed by Drs. Lewis Schanker, Dominick Tocco, Bernard Brodie, and Adrian Hogben in two reports published in the Journal of Pharmacology and Experimental Therapeutics. The papers report the relative rate of absorption of a large number of drugs which the investigators perfused in solutions through the large and small intestines of anesthetized rats.

Since the ability of acidic and basic organic substances to dissolve in fat is determined by their degree of dissociation, the pattern of drug absorption which was found can be explained by the concept that the membrane separating the intestinal contents from the blood stream has the properties of an oily barrier which admits drugs in their undissociated form while restricting passage of dissociated forms.

By providing an understanding of the factors that govern intestinal absorption of foreign compounds, such information furnishes useful guidelines for predicting the rapidity and degree of absorption of new drugs.

BANANAS FOUND TO CONTAIN LARGE AMOUNTS OF SEROTONIN AND NOREPINEPHRINE

Bananas have been found by NHI investigators to contain large quantities of serotonin and norepinephrine. These amines have great clinical and biochemical interest but have usually been associated with animal, rather than plant, physiological functions.

Following a report by Dr. J. A. Anderson and his associates of the University of Minnesota that animals and men showed an increased excretion of 5-hydroxyindoleacetic acid (5HIAA) in the urine following ingestion of bananas, Drs. T. Phillip Waalkes and Albert Sjoerdsma of the National Heart Institute's Laboratory of General Medicine and Experimental Therapeutics and Drs. Cyrus R. Creveling, Herbert H. Weissbach and Sidney Udenfriend of its Laboratory of Clinical Biochemistry investigated in an effort to explain the rather surprising finding.

They found that bananas contain large amounts of serotonin, an average of 3.7 milligrams of serotonin in each, although Anderson

and his co-workers had been unable to demonstrate serotonin (the immediate precursor of 5HIAA) or serotonin precursors in bananas.

Further analyses revealed that bananas also contain surprisingly large quantities of both norepinephrine and dopamine. Though 5-hydroxyindoles have been found in plants previously, this is the first demonstration of norepinephrine in plant material.

The findings of these studies have had an immediate practical application. They indicate that bananas should not be fed to patients in the period preceding the collection of their urine for diagnostic tests for malignant carcinoid and pheochromocytoma, because this might lead to false positive diagnoses of these tumors.

The new findings have also encouraged the NHI group to administer large amounts of serotonin and norepinephrine orally to patients. Study of metabolites excreted in the urine may shed further light on the enzymatic processes involved in the mechanism of drug actions and of the regulation of blood pressure.

HEMATOCRIT RATIO SEEN AS FACTOR IN SUSCEPTIBILITY TO HEMORRHAGIC SHOCK

Susceptibility to irreversible hemorrhagic shock is greater in dogs with either unusually high or low hematocrit ratios, Drs. Jack W. Crowell, Sara H. Bounds, and W. W. Johnson of the University of Mississippi Medical Center have found. Results of their NHI grant-supported investigations show that the hematocrit ratio--the proportion of red blood cells to blood plasma--was important as a factor in the time required to produce a state of irreversible hemorrhagic shock in 65 to 67 dogs studied.

In addition to the range of natural variations in hematocrit ratio present in the dogs, the investigators obtained variations by administering acetyl phenylhydrazine, by hemorrhage, and by transfusing red blood cells. Experimental shock was then produced in the animals by bleeding (by catheter through a tube to a bottle reservoir) to the degree necessary to lower the arterial pressure to 30 millimeters of mercury. When blood flow in the tube ceased and reversal of flow occurred, the withdrawn blood was reinjected.

The time required for the reversal to occur, the indicator of development of irreversible shock, was longer in dogs with average hematocrit ratios. The investigators found that an increase in the hematocrit ratio from 12 to 35 increased the resistance of the animals by a factor of approximately 5. However, increasing the ratio above 35 decreased resistance to irreversible shock.

Reporting their findings in the American Journal of Physiology, the investigators conclude that abnormal hematocrit ratios may increase susceptibility to shock by (1) altering the oxygen-transport capacity of the circulation or (2) changing the blood's buffer capacity, its ability to neutralize and eliminate tissue acidity. They also point out that although the potential oxygen-carrying capacity of the blood is increased in the higher hematocrit ratios, the greater viscosity decreases blood flow and thus reduces the total amount of oxygen carried to the tissues.

BASIC DIFFERENCE BETWEEN CONDITIONS OF RIGHT-SIDED HEART IS OUTLINED

On the basis of evidence from three patients studied at the Heart Institute and 116 cases in the medical literature, Dr. Robert P. Grant of NHI's General Medicine and Experimental Therapeutics Branch has outlined the fundamental distinction between dextroversion of the heart and mirror-image dextrocardia.

Understanding the nature of dextrocardia sheds light on associated congenital abnormalities, from the standpoint of their genesis as well as their diagnosis.

The most familiar type of right-lying heart is mirror-image dextrocardia, in which all cardiac structures are simply rightward mirror images of the normal heart. A similar reversal of left-to-right orientation usually occurs in the abdominal organs in this benign condition, and all structures are usually normal in other respects.

In dextroversion the blood-receiving, or atrial, chambers of the heart retain their normal orientation, but the ventricles are rotated to the right, much like turning the page of a book. With this condition there is usually a defect in the septum or partition between the ventricles as well as a transposition of the two great blood vessels leaving the ventricles.

Abnormalities of abdominal organs were found in about a fourth of the autopsied dextroversion cases studied in the medical literature and in one of the three patients referred to the Heart Institute. But instead of being inverted and otherwise normal, as in mirror-image dextrocardia, these aberrations suggested an arrest of embryologic development at an early fetal stage, before the two sides of the body lose their primitive symmetrical relationships. The organs in dextrocardia are abnormally symmetrical--often the two lobes of the liver are the same size, and the gall bladder, stomach, duodenum, and pancreas tend to be centrally positioned. Additional primitive conditions, such as tri-lobed lungs and absence of the spleen, are

also found. The heart may show extreme variations little advanced beyond the stage of the single tube, as found in fish, and may have only one atrium, one ventricle, or one great arterial vessel.

Dr. Grant has reported these studies in the journal Circulation. "It is suggested," he writes, "that dextroversion is part of a potentially widespread developmental defect which takes place at a very early fetal stage when the body first begins to search out its normal asymmetry, when cardiac septation and cono-truncal differentiation start, when the splenic bud first appears, and when the ventricular loop is predominantly rightward in location. Depending upon the intensity and distribution of the abnormality, a wide variety of intra-cardiac and extra-cardiac anomalies and combinations of anomalies may result. Dextroversion of the heart is perhaps only one part of this syndrome."

POTENT ARTERY-DILATING SUBSTANCE FOUND IN NORMAL HUMAN URINE

Normal human urine has been found to contain a powerful artery-dilating agent that resembles nitroglycerine in its ability to increase the coronary blood flow to the heart muscle.

This finding was reported in Federation Proceedings by Drs. K. E. Sussman, S. J. Sarnoff, and L. C. Sarnoff of the National Heart Institute, Dr. R. B. Case of St. Luke's Hospital in New York, and Dr. R. Macruz of Sao Paulo, Brazil. The work was done while Drs. Case and Macruz were with the National Heart Institute.

Using a test preparation of isolated living dog heart, these investigators found that less than half an ounce (10 cubic centimeters) of normal urine produces 2 1/2 to 3 times the increase in coronary blood flow produced by .6 of a milligram of nitroglycerine, the usual dose of this drug taken by heart patients to dilate the coronaries and relieve their angina pectoris.

This artery-dilating activity was found to be largely lacking in urine from four patients with abnormally low blood pressure (hypotension) resulting from disorders of blood pressure-regulating mechanisms in the nervous system. The average artery-dilating activity from these hypotensive patients was less than 15% of the average activity from five normal control subjects, the investigators report.

The role of the artery-dilating agency in normal persons and the exact significance of its lack in the hypotensives are not yet known. But the findings suggest two alternative possibilities to the scientists. Its deficiency in patients who have a defect of the

central blood pressure-regulating mechanism could mean that the artery-dilating agency may play some part in the normal function of that part of the nervous system. On the other hand, changes in the output of the dilator substance may result from changes in the level of blood pressure--its lack in the hypotensives might be a response to their abnormal blood pressure, rather than an aspect of its cause.

These possibilities are being explored at the Heart Institute in the continuing research, which is intended to gain for medical science a better grasp of the mechanisms that govern pressure and flow in the arteries in the normal state and in diseases such as hypertension, hypotension, and coronary heart disease, in which health is threatened by disorders of pressure and flow.

THREE ASPECTS OF ELECTROLYTE TRANSPORT IN CELLS MAY BE GOVERNED BY SINGLE GENE

The transport mechanisms that govern the electrolyte balance of cells are being studied in the red blood cells of sheep by investigators in the Heart Institute's Laboratory of Kidney and Electrolyte Metabolism. Sheep are being used because they may be classified into two types, one with high potassium and one with low potassium red cells. A single gene seems to govern which type occurs in the individual sheep.

The findings of the NHI investigators, Drs. Daniel Tosteson and Joseph Hoffman, indicate that a single gene may also govern the activity of three different components of sodium and potassium transport mechanism in the red cells. There are 1) active transport, in which the potassium moves into and the sodium out of the cell against the natural electrochemical potential gradient, requiring energy from metabolism for the process; 2) passive diffusion, in which potassium and sodium diffuse according to their electrochemical potential gradients across the cell membrane; and 3) exchange diffusion, the obligatory exchange of an internal with the same kind of external electrolyte (e.g., sodium for sodium).

The investigators found that a large proportion of the sodium flux in both types of sheep red cells occurs by exchange diffusion. Passive permeability differs markedly in the two types, with the low potassium type having a higher permeability to potassium and lower to sodium than the high potassium type. The high potassium cells exhibited four times as much active transport of potassium as did the low potassium type, and active transport of sodium was found to be greater in the high potassium cells than in the low. The findings were presented at a meeting of the Society of General Physiology held at Woods Hole, Massachusetts.

The details of the mechanisms regulating the processes of potassium accumulation and sodium extrusion by cells are of much interest because these coupled processes are intimately related to the regulation of osmotic pressure in organs of secretion, to conduction in nerves and muscles, and to other fundamental physiologic processes. Active sodium extrusion and potassium accumulation are also vital for preventing the colloid osmotic swelling that threatens all cells.

TECHNIQUE ALLOWS DELAYED RECOVERY OF STREPTOCOCCI FROM THROAT SWABS

Dr. Nell Hollinger and Miss Lois Lindberg of the University of California have successfully attacked the problem of recovering live streptococci from throat swabs after several days lapse of time during transport to culture media.

The Berkeley investigators collect the throat material on dry non-absorbent dacron swabs or on filter paper strips which are then further dried during transport. The strep organisms remain alive, and are recoverable when the swabs or paper strips are placed in contact with conventional blood agar culture plates after as much as ten days in transit from the field to the laboratory.

The investigators, whose studies were aided by an NHI grant, describe their development of the technique in the American Journal of Public Health. Evidence necessary for the diagnosis of the infections that precede rheumatic fever often cannot be obtained because of the inadequacy of present culture techniques, they explain. These techniques require the immediate availability of media and facilities for culture, such as the plates of nutrient blood agar and incubation facilities, by which the streptococci, freshly swabbed from the throat, are grown without delay.

Past attempts at overcoming the problem of delay during transport to such facilities, had been based on an assumption that streptococci died in the swab because of drying or oxidation. Liquid, semi-solid, and solid holding media were therefore tried by various scientists, but none of the methods based on this assumption gained universal acceptance.

Searching the literature for evidence on which a better solution might be based, the Berkeley investigators found several pertinent papers, including a German report more than 30 years old. This report described recovery of streptococci from cotton swabs left under dry conditions in sun and wind or stored in the dark for as much as a year. Following such delays, the German investigator, H. M. Jettmar, found that it was necessary to pulverize the swabs

and scatter their dust over the culture plate to recover the organisms.

The Berkeley investigators surmised that the usual inability to recover the dry organisms might be due to their tenacious involvement in the absorbent fibers of the cotton swab. They adopted dacron swabs as a result of tests showing dacron to be less absorbent than cotton. With these, laboratory strains of streptococci were recovered on blood agar culture plates after three days' dry storage in a tightly closed tube. Adding a silica gel drying agent to the caps of the storage tubes improved recovery still further.

To test the promising laboratory findings, a pilot study was conducted in an outpatient pediatric clinic. The throat of each child was sampled with two dacron swabs. One of each pair was the control, and was immediately streaked across a blood agar plate and incubated, according to more conventional practice. The other swab of each pair was stored in a silica gel tube and then cultured after 48, 96, or 144 hours, to simulate periods of postal delay.

Of 143 throats sampled, 41 (29%) were found infected with beta hemolytic streptococci. Eight of these were revealed only by the delayed swabs--they were missed by the controls. The investigators attribute this apparent superiority of recovery after delay to the drying. This, they feel, may weed out other types of organisms which are picked up on the swabs and which sometimes spoil the diagnosis by crowding out the streptococci on the culture plates.

Techniques similar to those of the pilot study were employed by the investigators in a field trial involving 1,603 paired cultures. A graduate student at the University of California, Mr. Harry DeBoer, had meanwhile found that streptococci introduced from dacron swabs to dry filter paper strips, would remain viable for 2-4 days of storage, so "kits" of filter paper strips were also included in the field trials. The trials showed it was necessary to air dry the strip 3-5 minutes after streptococci were introduced from the swab and before the kit was stored or packaged for shipment.

The efficiency of the dacron swab method was confirmed in the field trials. In addition, a 90% recovery rate was found from material stored 2-10 days on filter paper strips, compared with only 74% recovery from their immediately cultured paired controls.

"The simplicity and efficiency of dacron swabs and filter paper strips, in the transport of pharyngeal material for recovery of beta hemolytic streptococci, are sufficient to warrant trial of either transport medium in epidemiological studies on the streptococci," the investigators conclude.

FAST, ACCURATE, CHLORIDE DETERMINATIONS POSSIBLE WITH NEW AUTOMATIC METHOD

An instrument and method have been developed at the National Heart Institute which make possible the determination of chloride in plasma, urine, other body fluids, and tissue extracts, with an unprecedented combination of speed, accuracy, and simplicity. Previous methods utilized either relatively time-consuming chemical techniques requiring special skills, or instruments which gave inconsistent results, particularly as applied to tissue extracts.

The advance is expected to facilitate investigations of the fundamental mechanisms of body water balance and distribution, acid-base balance, and nerve and muscle excitability which depend on normal concentrations of chloride. The instrument and method also show clinical promise in the study of the many diseases which involve abnormalities of chloride metabolism, such as cystic fibrosis of the pancreas, Cushing's syndrome, Addison's disease, diabetes, renal diseases, meningitis, diarrheal diseases, dehydration, and the edemas of heart, kidney and liver disease.

The advance was announced in The Journal of Laboratory and Clinical Medicine by Dr. Ernest Cotlove of the Laboratory of Kidney and Electrolyte Metabolism and Mr. Hillary Trantham and Dr. Robert Bowman of the Laboratory of Technical Development. The instrument provides automatic electrically-metered titration of chloride throughout the range of chloride concentrations found in biological fluids. Consistent reproducibility (within .5% error) has been obtained at the Heart Institute in the course of thousands of chloride determinations using samples from humans and animals.

The instrument can be operated by individuals with little technical training. The turn of a switch starts both a timer and the electrolytic release of silver ions into the solution from an electrode of silver wire. The silver ion combines with and precipitates the chloride at a constant rate (determined by the value of the constant current). After all of the chloride has been precipitated, the silver ions continue to accumulate in the sample, causing an abrupt rise in current across a pair of silver "indicator electrodes" which activates a meter-relay circuit, stopping the timer. This reveals the chloride content of the sample, since the total amount of silver ions generated at constant current is directly proportional to the elapsed time, according to Faraday's law. The time required for each titration can be varied by selecting different levels of electrolytic current, and extreme precision can be sacrificed for speed in order to reduce the time of the determinations to 15 seconds or less.

Determination of substances in solution by such automatically timed electrolytic titration methods is not new. The unique accuracy and versatility which distinguish the NHI instrument result from its particular indicator electrode and relay systems. Various indicator

electrodes of more conventional sorts were tried (platinum, silver-plated platinum, gold, and tantalum) and the results were found inconsistent and unsuitable. The silver indicator electrodes have given consistently accurate results in the course of thousands of determinations.

MODEL SYSTEM DEVELOPED FOR LABORATORY STUDY OF UNESTERIFIED FATTY ACID

Findings by NHI investigators have made possible the development of a model system for the *in vitro* study of chemical and physiological factors influencing the release of unesterified fatty acid (UFA) from body fat depots.

Evidence published last year from NHI studies in patients by Dr. Robert S. Gordon and Miss Amelia Cherkes strongly indicated that unesterified fatty acid (UFA) is released into the blood from stored fat to satisfy the body's energy requirements during the fasting state. The studies showed that the level of UFA in the circulation of humans is increased by fasting and by injections of epinephrine, and is decreased by a meal (or the administration of glucose) and by insulin.

More recently Dr. Gordon and Miss Cherkes have demonstrated that UFA can be released from fragments of adipose tissue incubated *in vitro*. The recent findings support the earlier conclusion from studies of circulating UFA in man that adipose tissue is the source of the UFA and that the transport of UFA out of fat depots accounts for the mobilization of stored fat to supply the organism during fasting.

Adipose tissue used in the *in vitro* studies consists of the epididymal fat bodies of young male rats. These paired fat bodies are found attached to the testis in the form of thin leaves of fat, which do not require the usual slicing or mincing preparation for incubation. One fat body of a pair from a particular animal serves as a control for comparison with the behavior of the other under the influence of a particular agent being tested. It seems likely, the investigators point out in *Proceedings of the Society for Experimental Biology and Medicine*, that this *in vitro* system constitutes a satisfactory model for the study of chemical and physiological factors influencing UFA production.

Studies with such a model system may provide insights into the nature of physiological mechanisms regulating the accumulation and distribution of body fat, and into some of the disorders and irregularities of fat metabolism associated with such diseases as diabetes and atherosclerosis. Findings made with such a system may also be applicable in future studies of the mode of action of compounds which are of clinical interest because they promote the gain or loss of body weight.

DIBENAMINE FOUND TO BLOCK TOXIN RESPONSIBLE FOR IRREVERSIBILITY IN SHOCK

Dibenamine has long been known to protect against the development of irreversibility to transfusion in hemorrhagic shock. The recent discovery of a toxin present in dogs and rabbits in hemorrhagic shock prompted Drs. F. G. Smiddy, D. Segel, and J. Fine of Beth Israel Hospital and Harvard Medical School, Boston, to investigate a possible relationship between this poison and the protective action of dibenamine.

Their experiments on rabbits, which were aided by a National Heart Institute grant, have been reported in the Proceedings of the Society for Experimental Biology and Medicine.

Dibenamine, given to animals bled to a state of reversible shock, was found to enable them to withstand transfusions of toxin-containing blood from irreversibly shocked donors, a procedure invariably fatal to recipients not protected by the drug.

Of ten dibenamine-protected rabbits, six recovered completely from shock following transfusion with toxic blood. Of the four that died, three lacked the hemorrhagic lesion in the gut that uniformly results from the bacterial toxin.

"This experiment demonstrates that dibenamine blocks the action of the toxin, and thereby not only preserves the responsiveness of the circulation, but also prevents the hemorrhagic lesion in the bowel wall, which is characteristic of irreversible shock," the investigators conclude.

ORIGIN OF BODY MECHANISMS FOR DISPOSAL OF FOREIGN SUBSTANCES IS TRACED

Research findings by scientists of the National Heart Institute's Laboratory of Chemical Pharmacology have shed light on the evolutionary development in the body of protective mechanisms that chemically alter foreign compounds and are responsible for the inactivation of most drugs. The findings, reported in Federation Proceedings by Drs. B. B. Brodie, R. P. Maickel, W. R. Jondorf and L. E. Gaudette, indicate that as animals evolved from aquatic to terrestrial forms, special mechanisms for disposing of foreign substances came into being, culminating in the highly efficient liver microsome enzyme systems in mammals.

Heart Institute investigators had previously discovered these enzyme systems which are found in liver microsomes, tiny particles in liver cells. Their studies suggested that the microsomal enzyme systems were developed in the liver of ancestral species as a way of eliminating

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useless or harmful substances taken into the body with food. Hundreds of compounds of no nutritive value to the animal are swallowed with food and absorbed into the blood, and more are made by the bacteria of the digestive tract.

Many of these compounds would be toxic to the animal if permitted to accumulate and thus must be disposed of. However, the kidney, the major route for disposal of waste material, has a limited ability to remove fat soluble materials from the body. Since many of these foreign substances are fat soluble, they would leave the animal only very slowly unless they are first made less fat soluble by some sort of chemical change. Many of these chemical changes can be classified as oxidation. These mechanisms which function to rid the body of foreign substances are obviously necessary for survival.

In tracing the development of these systems in lower forms of animals, the investigators found that the mechanisms which convert fat soluble substances to an excretable form developed to fill a need created by changes in the environment of the animal. For instance, fish lack these mechanisms. But these animals do not need them for they possess gills which readily dispose of fat soluble substances by diffusion into their watery environment. However, as land animals developed, water conservation became a major problem. The subsequent development of less permeable skin membranes went hand in hand with the need for enzyme systems capable of chemically changing foreign compounds into forms which the kidney could excrete.

A group of compounds (including amphetamine, methylanaline, aminopyrine (pyramidon) and hexobarbital (Evipal), were found to be excreted by mammals only after oxidation to less fat soluble derivatives. Many species of fish and amphibia including salamanders and frogs lack mechanisms to alter these drugs chemically, but excrete them unchanged (through the gills of the fish and the skin of the salamanders and frogs). The ability to oxidize foreign compounds first appears in the reptiles from which it was inherited by the mammals and birds.

Toads, while closely related to frogs, lead a terrestrial life and have skins which are very poorly permeable to water. Consequently, it is not surprising to find that the toads can oxidize drugs. The mechanisms used by the toads, however, are different from those found in mammals.

Thus the emancipation of animals from the sea is associated with mechanisms that oxidize foreign compounds to excretable derivatives. Nature has had to solve this problem a number of different times with reptiles, with toads and with insects, for example. Each time she has solved the problem differently.

Phylogenetic studies were also made of the mechanisms that make foreign compounds known as phenols less fat soluble by conjugating or joining them with glucuronic acid or with sulfate. The investigators found that these processes are absent in fish; they first appear in frogs and other amphibia. They are also absent in tadpoles, the aquatic juvenile form of frogs.

SIMPLE METHOD DEVELOPED FOR MEASURING MONOAMINE OXIDASE INHIBITION

The clinical application of drugs which inhibit the enzyme, monoamine oxidase, has been stimulated by recent work with iproniazid. This drug, known to be a potent monoamine oxidase inhibitor, is being used in psychiatry in treating depressed mental states. It has also been reported to be effective in some cases as a hypotensive agent and in the treatment of angina pectoris. Evidence of liver damage and some deaths have been associated with the administration of iproniazid, however, and recent studies have been directed towards the exploration of other monoamine oxidase inhibitors.

An important advance in this field has been the development by National Heart Institute scientists of a simple method for the measurement of monoamine oxidase inhibition in man. The test stems from previous NHI findings indicating that monoamine oxidase is the enzyme primarily responsible for limiting the action of serotonin in the body. In the test, serotonin is given orally in doses of 20 milligrams two hours after administration of a presumed monoamine oxidase inhibitor. Urine is subsequently collected and assayed for 5-hydroxyindoleacetic acid, the end product of serotonin metabolism. Reduction in the percentage of conversion of serotonin to 5-HIAA reflects the action of a compound in inhibiting monoamine oxidase. The test has been shown to be sensitive and is being used at the Heart Institute in the evaluation of monoamine oxidase inhibition in man.

A report on the method, by Drs. Albert Sjoerdsma and Louis Gillespie, Jr. of the Clinic of General Medicine and Experimental Therapeutics and Dr. Sidney Udenfriend of the Laboratory of Clinical Biochemistry, has been published in the journal Lancet.

Items of Interest on Research Studies Conducted and Supported by

the National Institute of Allergy and Infectious Diseases

ALLERGY-IMMUNOLOGY

ALLERGENS PERSISTING IN BODY MAY BE LONG-TERM SOURCE OF ALLERGY OR IMMUNITY

Long-term presence of antigen in the body as a source of antibody formation for long-term immunity or hypersensitivity is being studied by Dr. Dan H. Campbell, professor of immunology and Dr. Justine Garvey, chemist, at the California Institute of Technology, Pasadena. The research is supported by the National Institute of Allergy and Infectious Diseases, the Rockefeller Foundation and the Office of Naval Research.

In work reported in the recent Bela Schick anniversary number of the International Archives of Allergy and Applied Immunology, the investigators "tagged" cow albumin with radioactive sulphur and injected this antigen into rabbits. After seven days almost all the antigen had been eliminated. Large amounts were detected in the urine. However, 500 days after the injections, radiation counters still detected some of the antigen material in the rabbit livers. Although the amount was small, it was equal to several thousand molecules of antigen for every liver cell. Dr. Campbell theorizes that this amount is sufficient to cause reactions, although the role of the long-lasting material has not been established. If this persistence of antigen contributes to prolonged allergic reaction, a means might be found to flush out the foreign material and eliminate the sensitivity.

When the long-lived antigen is extracted from the liver of experimental animals, it is found in solution with ribose-nucleic acid. The body used this RNA to build protein. Apparently the injected antigens fragment and combine with RNA. This combination could now act as a template, stamping its pattern on protein globules to form specific antibodies.

The Caltech immunologist hypothesizes that persistence of antigens in the body may explain why small booster shots can maintain immunity after immunization has been established by vaccination with a heavier dose of vaccine (antigen). The antigen-RNA in "hibernation" provides ready templates for stamping out antibodies, according to this theory. These antibodies may give immunity or cause allergic reactions. The persistent antigen (pollens involved in hay fever and asthma are also believed to persist as antigen-RNA combinations) may also be involved in immune tolerance, anamnestic or secondary responses, and perhaps the stabilization of small amounts of antibody in tissue spaces.

EXPLORE BACTERIAL ALLERGY USING PREVALENT STAPHYLOCOCCI AS EXPERIMENTAL MODEL

While many investigators explore resistance of staphylococci to antibiotics, another aspect of these bacteria--their possible allergenic effect--is under study by NIAID grantees S. M. Beiser and Murray Dworetzky and associates at Columbia and Cornell Universities. Because of their ubiquity, staphylococci have been foremost among suspects.

In laboratory studies, the investigators believe they have demonstrated in pooled human gamma globulin the staphylococcal antibodies significant in allergy. They also reported that invasiveness or pathogenicity of a strain of staphylococcus may be associated with its ability to sensitize humans, as indicated by bands around reactive sites of various staphylococcal filtrates in agar preparations of pooled human gamma globulin.

In additional research, skin response to polysaccaride fractions from staphylococci were more frequent in allergic than in normal persons.

Protein fractions produced hypersensitivity in guinea pigs. A segment of intestine from a guinea pig which had received sensitizing staphylococcal injections was isolated and attached to a kymograph to give a written record of longitudinal contractions of the intestine tissue, as an indication of allergic response. The segment was challenged with various fractions of staphylococci in the presence of pooled human gamma globulin. (The gamma globulin was found to suppress any primarily toxic reaction.) Nucleoprotein fraction from S. albus was more allergenic than that from the S. aureus strain. A more comprehensive understanding of staphylococcal diseases should derive from continuing studies of their allergic aspects.

UNION OF INSULIN WITH NON-PRECIPITATING ANTIBODIES CAN NOW BE MEASURED DIRECTLY

One problem that has puzzled immunologists for many years may be on its way to solution: how to measure the union of antigen with antibody directly. So long as the result of this union is apparent, e.g., agglutination, precipitation, hemolysis, or the like, it can be measured indirectly. One of the cases in which there is no such reaction to measure is the production of antibody responding to and combining with insulin. The existence of this nonprecipitating antibody has long been surmised from occasional local reactions to insulin and because of some of the biologic effects which the plasma of insulin-treated patients has been known to possess. Electrophoretic studies proved that insulin combines with gamma globulin.

Joseph H. Skom and David W. Talmage of the University of Chicago, have found a means of directly measuring the binding of insulin by the antibodies of human serum. Their work was supported by the National Institute of Allergy and Infectious Diseases.

The investigators showed first that insulin was soluble in anti-human gamma globulin (prepared by injecting human gamma globulin in rabbits). It was further shown to be soluble in human gamma globulin and a supernatant of the two. They then tagged insulin with radioiodine and added it to human serum. Finally, to precipitate the responding antibodies, they introduced AHGG. The insulin thus bound to the antibodies naturally followed the precipitate and could be measured by its associated radioactivity.

In subsidiary experiments, the investigators demonstrated that the precipitation of the globulin does not increase the binding of insulin nor does it result in dissociation of the insulin from the globulin.

DIFFERENT SERUM PROTEINS IN ANIMALS OF SAME SPECIES MAY BE HUMAN ALLERGY FACTOR

It has generally been assumed that serum proteins within the same species are antigenically identical and that the production of antibodies in one individual to the serum proteins of another of that species would be impossible. A recent study by Oudin of the Pasteur Institute in Paris demonstrated, however, that rabbits can be grouped on the basis of antigen-antibody reactions between sera and antisera of the same species.

Dr. Sheldon Dray and Miss Glendowlyn O. Young of NIAID's Laboratory of Immunology have now extended the work of Oudin. They have found that various components of "normal" rabbit sera, such as alpha, beta, and gamma globulin, induce the formation of antibodies which were demonstrated by precipitin tests. These investigators also detected these induced isoantibodies by the passive cutaneous anaphylactic test.

The rabbits were injected with normal rabbit sera plus paraffin-oil type adjuvants. On the basis of the presence or absence of precipitin bands, the 90 rabbits tested could be separated into 13 groups, or into 30 by taking the varying number of bands into consideration.

This antigenic difference in the serum proteins from various individuals of the same species is a basis for the study of how such proteins are inherited. It is pertinent, moreover, to immune tolerance in tissue grafting. The findings of Dray and Young of serum groups analogous to blood groups provides a new lead--the possibility that an allergic or immunologic mechanism involving the serum proteins may be a factor in some of the transfusion reactions in man.

LOOK FOR CLUES TO ALLERGY IN POTENT, SHOCK-PRODUCING GUINEA PIG GLAND SECRETION

The coagulating gland is a small structure whose secretion contains an enzyme capable of coagulating the fluid of seminal vesicles. The gland occurs only in a few species of animals, namely in guinea pigs, mice, rats, hamsters; but most species, including man, horse, swine, dog, and the rabbit, do not have this gland.

While studying the antigenic properties of the prostate, NIAID's Dr. Jules Freund and his former associate, G. E. Thompson at the Public Health Research Institute of the City of New York, Inc., reported discovering that secretion of the coagulating gland from guinea pigs is highly toxic to guinea pigs and particularly so to rabbits. The intravenous injection of 0.002 ml. of this secretion causes hypotensive shock in the rabbit, the animals dying after nystagmus, dyspnea, hypothermia, cyanosis and other shock symptoms. Intracutaneous injection produces swelling and discoloration, first red, then purple. The induction of edema suggested that the most significant aspect of the toxicity of the coagulating fluid may be the alteration of the permeability of small blood vessels.

The field of vascular permeability plays an important role in the early phase of inflammation, infectious or non-infectious in origin. In many and diverse manifestations of allergy the increase of vascular permeability is one of the most conspicuous aspects.

Dr. A. A. Miles, Director of the Lister Institute of Preventive Medicine in London, made many contributions to the study of the early phase of infection of the skin. Furthermore, he and his associates discovered that the serum of normal guinea pigs contains a factor which enhances the vascular permeability of inflamed areas. A cooperative study in a mutually interesting field of research was started between the Lister Institute and the National Institutes of Health. A report in Nature gives an account of biochemical and biological data obtained mostly by methods the English

investigators developed during recent years. The gland secretion was purified by starch electrophoresis. Of the 5 fractions obtained, two, namely Fractions I and II, contained the permeability factor and were free of the coagulating enzyme. Fraction I had an electrophoretic mobility slightly higher than that of serum albumin, was homogenous in the ultra centrifuge and had a molecular weight of about 70,000. It consists mainly of protein but on hydrolysis yields a small amount of carbohydrate. It contained a strong p-toluene-sulphonyl arginine methyl esterase.

The permeability increasing potency was measured in terms of blue exudation into the skin of animals with a blue dye in the circulation and is expressed in blueing doses per milligram. Tested in the rabbit, the original fluid from the guinea pig gland had a blueing dose per mg. 200,000 units and Fraction I 600,000 (for comparison histamine 37,000 units). Fraction I reproduced the hypotensive shock as seen after the injection of the original coagulating gland secretion. It is of interest that Fraction II had little systemic toxicity for guinea pigs and rabbits but its coagulating potency was higher than that of the original fluid. Ten to 16 mg/kgm of Fraction II in the rat produced certain symptoms including edema of the snout. The coagulating gland of species other than the guinea pig contains very little toxic material.

The main toxic material in the coagulating gland secretion of the guinea pig is in the Fraction I which contains little if any coagulating enzyme. On weight basis the potency in the guinea pig is nearly twice as high as that of histamine; in the rabbit it is far higher. On molar basis the potency of Fraction I for rabbits is about 9000 times that of histamine.

This fraction is toxic for the homologous animal, mainly for the small blood vessels. This may be irrelevant to its function in the external secretion of which it is a part. Its pharmacology may provide a clue to the mode of action of the enzyme-like protein permeability factors present in the blood and tissue fluids.

SOME STEROIDS AND TRANQUILIZERS PROTECT SENSITIZED MICE AGAINST SHOCK REACTION

Dr. Carl T. Nelson and associates at Columbia University College of Physicians and Surgeons report that out of 34 compounds tested, five steroids, three tranquilizers, and one serotonin antagonist were found to exert maximum protection against shock reaction in sensitized mice. The investigation is supported by the National Institute of Allergy and Infectious Diseases.

The steroids which protect mice against fatal anaphylaxis are prednisolone, prednisone, fluoro-hydrocortisone, hydrocortisone, and cortisone. The efficacy and potency of the corticosteroids are related to the degree of sensitization achieved--larger doses

of the drugs are needed for protection as the sensitization increases.

In contrast, the tranquilizing agents--chlorpromazine, promazine, and reserpine--protect the mouse from shock reaction even at levels of maximum sensitization.

Small doses of the serotonin antagonist, lysergic acid diethylamide, (LSD) administered one to two hours prior to challenge also protect against anaphylaxis.

Other compounds--meprobamate, sedatives, antihistaminics, inflammatory agents, diuretics, and predominantly sodium-retaining steroids--although tested in relatively high dosages--are not effective in preventing anaphylactic shock in mice.

The protection exerted by the various compounds was gauged by survival of sensitized mice given these drugs prior to challenge compared with incidence of fatal shock reaction in untreated mice.

The finding in this study that compounds like LSD, chlorpromazine and reserpine are active in preventing anaphylactic shock indicates, the researchers say, that this protective property is not confined to steroids and further investigation may disclose an essential common denominator in terms of pharmacologic action.

"Effective substances for protection against anaphylaxis," the investigative group says, "appear in general to be primarily natriuretic rather than sodium-retaining, capable of simulating marked increases in adrenal hormone levels, adrenergic in terms of epinephrine enhancement (but not epinephrine itself), central nervous system tranquilizers exhibiting marked effects on behavior, and antagonists of serotonin. Compounds acting primarily as vasoactive agents, antihistaminics, sedatives, analgesics, anti-inflammatory agents and diuretics are not effective in protecting mice from anaphylaxis."

ANTIBODIES FOUND TO PERSIST IN VOLUNTEERS GIVING BLOOD THROUGH PLASMAPHERESIS

Plasmapheresis is a method through which, according to Dr. Joseph Smolens and associates at the Children's Hospital, Philadelphia, a person may safely donate blood plasma as often as 50 times a year. Results of employing plasmapheresis on volunteer donors (Philadelphia park guards) have now been observed for about three years by the NIAID grantees.

They used the "ADL-Cohn Blood Fractionator" in performing plasmapheresis 598 times during the first year. Two donors gave weekly; the others of the 23 volunteers gave blood plasma bi-weekly

for 40 weeks. The fractionator employs a closed (sterile) system centrifugation principle. The lighter plasma component of blood is retained, sealed in plastic bags, while the heavier part, including the red and white cells, is put back into the donor almost immediately. The plasma proteins are regenerated in the donor within 16 to 18 hours to replace those withdrawn.

In checking upon the safety of this procedure, red and white blood cell and platelet counts, hemoglobin, hematocrit, percent nitrogen, reticulocyte counts, white cell differentials, bone marrows, prothrombin clotting times, paper electrophoreses and other tests were made at various times. All were within normal limits.

The feasibility of building up in the donor plasma antibodies of possible use to a recipient has most recently been examined. Past experience demonstrated that it is possible to achieve very high antibody titer in about one of five persons by injections of the corresponding antigen.

During the 5th, 6th, and 7th visits, groups of donors were given three intramuscular injections of pertussis vaccine, $\frac{1}{2}$ ml. each; and three intramuscular injections of mumps vaccine, $\frac{1}{2}$ ml. each injection. A control group received no vaccine.

The pertussis and mumps antibodies persisted at high levels over the entire two-year period. This was unexpected, the grantees comment. They advance a chemical theory to explain the replacement of original antibody during the first year when removal of some products for transfusion apparently gave impetus to cells which synthesize antibody. There is no immediate explanation, they note, for the retention of the high antibody levels during the second year when no plasmapheresis was done. This phenomenon will continue to be investigated.

The investigators believe that these studies are demonstrating the feasibility of plasmapheresis as a practical means of stockpiling large quantities of plasma from carefully screened donors and that "ready-made" antibodies against a number of diseases may be provided in the plasma through prior injections of the donors. They are studying a third type of antibody, tetanus antitoxin. Human antitoxin, if feasible, would be superior to and safer than horse globulin antitoxin commonly used today and occasionally involved in allergic reactions of varying severity.

TRANQUILIZER PROVES EFFECTIVE IN TREATMENT OF CHRONIC URTICARIA

Although treatment of allergy with a tranquilizer is generally unsatisfactory, Drs. Alan R. Feinberg and associates of the Allergy Research Laboratory, Northwestern University Medical School, report one

exception--the relief of chronic urticaria and dermographia by hydroxyzine (Atarax). A research grant from the National Institute of Allergy and Infectious Diseases supported their work.

In treating 60 patients, some with multiple symptoms, the investigators gave an initial dose of 10 mg. Atarax in capsule 3 times a day, raising this to 25 mg. in the absence of appreciable relief.

Treatment of either atopic or contact dermatitis and hay fever was not completely satisfactory, but better results were obtained in vasomotor rhinitis. Six of 10 patients got some relief. Of 13 with asthma, 7 received fair to good relief. One of 3 patients with dermographism had excellent relief and the other two good relief.

Most striking results were observed in patients with chronic urticaria of undetermined etiology. Fifteen of 17 patients experienced complete relief, the remaining 2 showing improvement. In several of the patients relief lasted only a few weeks. In others it was permanent and could be maintained on a 10 mg. dose given as infrequently as every 24 to 72 hours. By contrast with chronic urticaria patients, only one of four with the acute form gained any real relief.

Of the 60 patients, the investigators noted side effects in 11. But where they obtained good therapeutic results, they found it unnecessary to discontinue the use of the drug.

TWO DIFFERENT TYPE ANTIBODY SUBSTANCES FOUND IN SERUM OF PERSON HYPERSENSITIVE TO ANIMALS

In a clinical and serological study of an individual extremely sensitive to animals, Dr. Carl E. Arbesman of the University of Buffalo, demonstrated a sensitizing and a neutralizing antibody in the patient's serum. Dr. Arbesman is a grantee of the National Institute of Allergy and Infectious Diseases.

It is well known that animal epidermals are a cause of clinical allergy. The most common ones that provoke allergic symptoms are those of domestic animals--the cat, dog, horse, and chicken. Rabbit hair, wool, and cow epidermal also are frequent offenders; and guinea pigs, rats, etc. have occasionally been the causative factors of allergic reactions in laboratory workers.

The patient studied by this investigator manifested severe reactions to white mice while working with them in the laboratory and, because of his extreme clinical sensitivity, a thorough study of this case was deemed advisable.

Intradermal skin tests were performed, using mouse, rat, horse, dog and cat danders and sera. The reactions to the sera and the corresponding epithelial extracts were essentially parallel and differed only in intensity. Mouse dander and serum produced the strongest reactions of all the animal danders and sera tested.

Passive transfer studies were carried out in 12 healthy, non-allergic male medical students and four allergy patients who gave negative response to the antigens being used. The patient's serum was injected into several sites on the recipients and these sites were then tested intradermally with various antigens 24 hours later. Controls were established by substituting saline for the sensitizing serum and the antigen and injecting it into one site.

Both the direct skin tests and the passive transfer study showed that the mouse and rat sera gave the strongest reactions. Dog and guinea pig sera were also important antigens. Reactions to hog, beef, and rabbit sera were only weakly positive and were negative in quantitative passive transfer studies.

The antibody in the patient's serum responsible for passive transfer of sensitivity to mouse sera was found to be heat labile and was completely destroyed after heating at 56°C. for 30 minutes or longer.

Hemagglutination tests were employed to demonstrate "antibodies" against animal danders and sera in the patient's serum. Most of the danders linked to human red blood cells were agglutinated by the allergic individual's serum. Dog and beef danders were the exceptions, but their respective sera provided positive hemagglutination. The wide range of animal species to which reactions could be manifested with the patient's serum was startling, the investigator reported. All mammalian species tested could be shown to react antigenically, with the exception of human serum. Attempts to find species giving negative tests led to the inclusion of sera from animals with which the person had no known contact, such as lions, tigers, elephants, and coyotes. Only avian and reptilian sera failed to agglutinate serum-linked erythrocytes.

The substance in the patient's serum capable of producing clumping together of antigen-linked red blood corpuscles was thermostable and was considered different from the patient's serum reagin antibody.

NEW HORMONE DISPLAYS HIGH POTENCY IN TREATMENT OF ALLERGIC CONDITIONS

Triamcinolone is a new and highly potent antiallergic hormone that produces therapeutic effects on one-half the dosage necessary for prednisone.

This conclusion is based on a 10-month study of patients at the Allergy Research Laboratory, Northwestern University Medical School by Drs. Samuel M. Feinberg, Alan R. Feinberg and Elmer W. Fisherman. The investigation was supported by a research grant from the National Institute of Allergy and Infectious Diseases.

The investigators administered the drug to 70 patients with allergies such as asthma, hay fever, urticaria, and a variety of dermatoses. Their major questions concerned the relative potency of the drug and its advantages or disadvantages as compared with other drugs. They found 6 mg. to be the average daily maintenance dose for triamcinolone, as compared to 10.6 mg. for methylprednisolone, and 12 mg. for prednisone.

In general, the doctors report that it behaves like other anti-inflammatory drugs with respect to side effects. One intriguing action, however, is the marked diuresis and sodium removal associated with its administration. Patients increased their urinary output and lost weight when switched to triamcinolone therapy.

The investigators considered the sodium removal an advantage in treating patients with cardiac disease or hypertension. They speculated, however, whether sodium depletion might not reach the handicapping stage in certain cases.

One disadvantage of this drug is an associated syndrome consisting of a sometimes severe headache, dizziness, drowsiness and weakness. This occurred in 12 out of the 70 patients treated. In some cases the effects were severe enough to force withdrawal from the drug. These symptoms disappeared in one to three days after another steroid hormone was substituted.

Loss of weight for a reason other than diuresis was fairly common with the administration of the drug. Unlike other hormones, it does not stimulate the appetite and may even depress it.

VIRUS DISEASES

HEMADSORPTION TEST LEADS TO ISOLATION OF TWO NEW RESPIRATORY VIRUSES

Eventual control of the common respiratory diseases depends on determining their causes. Despite recent notable advances in identifying several viruses as agents responsible for certain respiratory illnesses, the causes of many of these diseases remain to be found. It is becoming increasingly clear that the "common cold," certain of the pneumonias, and other such illnesses may be due to a variety of viral agents and depend on many factors, such as the season of

the year and the age of the patient. In January 1958 two new viruses were reported in children with respiratory illnesses. These agents were uncovered by Dr. Robert M. Chanock and associates of the National Institute of Allergy and Infectious Diseases, in collaboration with Dr. Robert H. Parrott of the Children's Hospital, District of Columbia, and Dr. B. E. Andrews, a Wellcome Foundation researcher.

They reported that 41 viral agents having influenza-like characteristics were recovered in monkey kidney tissue cultures from throat swabs during a preliminary study of infants and young children with respiratory illnesses who were patients in Washington area institutions. The majority of these viruses, however, were not identified by the standard influenza antisera used for typing.

Preliminary clinical and epidemiological observations indicated that these newly discovered agents may be responsible for a proportion of common acute respiratory illnesses in children. Serologic data suggest that their contribution to respiratory diseases in children may be substantial. Preliminary surveys for antibodies against the new agents in the serums of 55 adults, nearly all young males, showed that all had neutralizing antibodies to the first of the two types of new virus and 39 had neutralizing antibodies to the second type.

The discovery of the new viruses grew out of tests with a laboratory method developed by NIAID for rapid diagnosis of influenza. The new agents are called hemadsorption viruses after the hemadsorption test.

The relationship of Type 1 hemadsorption virus to illness was indicated during examinations of throat swabs collected from 168 infants and young children with acute and feverish respiratory illness, and 82 children of approximately the same ages without respiratory illness. They were patients in Children's Hospital of the District of Columbia, D. C. General Hospital, and Georgetown University Hospital, Washington. Type 1 hemadsorption virus was isolated from 8 of those with illness (feverish pharyngitis, bronchiolitis, or pneumonia) but from none of the 82 persons without respiratory illness.

A number of virus isolations from nursery children at Junior Village, a D. C. welfare institution, also bore out a probable causative relationship between the Type 1 hemadsorption virus and respiratory disease. Of 27 Junior Village children with Type 1 virus isolation, 22 had fevers and various respiratory symptoms, including coughing. Most of the children appeared only moderately ill, in spite of the persistent fever and other manifestations of infection.

Strains of the other type (Type 2) hemadsorption virus were recovered from two children with croup hospitalized in Washington. Type 2 strain was also isolated from a croup patient hospitalized in Cincinnati. The virus was demonstrated in one of 28 throat swabs from croup patients there.

Serologic tests further demonstrated that both Types 1 and 2 hemadsorption viruses are present in Cincinnati as well as in Washington and suggest that this may be a common respiratory virus in many communities.

At least 70 newly recognized viral types representing several different groups have been described since 1948. The two newly isolated types of hemadsorption viruses of the myxovirus group are distinctly different from any of the other known viruses, although they have influenza-like characteristics and show minor relationships in guinea pig tests to mumps and Sendai (influenza D) viruses. The primary interest in searching for and characterizing new viruses is to determine their contribution to the respiratory diseases which are particularly prevalent in infants and young children. Also, it has been shown that vaccines can be developed to protect against certain viral respiratory diseases, once the causative agents are known.

HEMADSORPTION VIRUS SHOWN TO PRODUCE ILLNESS IN INOCULATED VOLUNTEERS

Inoculation of human volunteers has shown that type 2 hemadsorption virus can cause respiratory illness in adults as well as in small children. Serologic tests have indicated that hemadsorption viruses may be prevalent in many communities and may be responsible for a substantial amount of influenza-like or "cold-like" illnesses. In adults the illness produced is mild as it is in most children. However, in certain infants and small children disease of a more serious nature (such as croup) is not uncommon.

The research reported by Thomas E. Reichelderfer, Robert M. Chanock and associates of the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, and Thomas G. Ward of the Lobund Institute, University of Notre Dame, was performed with 32 healthy male volunteers. These men, from 21 to 46 years of age, were inmates at the Maryland State Board of Correction's Patuxent Institution at Jessup, Maryland.

The Patuxent volunteers were given the virus by spraying their throats and nasal passages. This agent was derived from an infant with acute laryngotracheobronchitis. /Acute bronchitis: marked by fever, difficulty in breathing, pain in chest, especially on coughing; laryngotracheobronchitis: involves inflammation of the larynx, trachea and bronchi./ Safety tests of the inoculum were

carried out in various tissue cultures, bacteriologic media and laboratory animals. No bacteria or contaminating viruses were detected.

A test of the blood of the volunteers before the study commenced indicated that seven of the men had no previous exposure to this virus; the remaining 25 had various levels of neutralizing antibody against the virus.

The volunteers were isolated in separate cells and had independent medical examinations each day by two physicians. Throat swabs taken from the men were tested in monkey kidney tissue cultures. Virus was recovered for the first time on the third day from 10 of the volunteers, with others shedding virus for the first time as late as the seventh day. The type 2 hemadsorption virus was recovered from 24 of the 32 volunteers. Twenty-five men developed a rise in antibodies against the agent.

When clinical illness failed to develop by the fifth day, the men were released from isolation. On the sixth day, however, six of the men reported to the infirmary complaining of respiratory illness and immediately all volunteers were returned to isolation. Examination of the other volunteers revealed that additional individuals had onsets of respiratory illness on this day. A total of 17 men developed respiratory disease. The onset of these illnesses coincided with the time when the greatest number of volunteers had virus demonstrable in their upper respiratory tracts. For the most part the illness was mild with prompt and uneventful recovery occurring within 2-3 days. The volunteers were released from isolation on the 11th day.

The most common symptoms of this "cold-like" illness were nasal obstruction and discharge, coughing, and sneezing. Less frequent were sore throat, headache, ocular complaints, vague chest pain after coughing, and chilliness. Aches, vomiting, anorexia and hoarseness were present in an occasional individual. Physical examination revealed a reddened edematous pharynx at the onset of clinical symptoms. A rhinorrhea that was serous and later became mucoid, and inflamed nasal mucosa and tonsils were the next most common findings. Four individuals had lacrimation and a mild conjunctivitis. Lung fields were clear by auscultation, percussion, and X-ray. Four men had a low grade fever (100° to 101°F.). White blood counts were within normal limits.

Eight days after the premature release of the volunteers from isolation a small outbreak of respiratory illness occurred in the general prison population. Eighteen individuals who were not in the study became ill with a mild "cold-like" illness without fever that lasted 2 to 3 days. Type 2 virus was recovered from 8 of the individuals in this group.

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RECOVERY OF COXSACKIE VIRUS FROM URINE OF INFECTED PATIENT SUGGESTS KIDNEY INVOLVEMENT

Dr. John P. Utz, National Institute of Allergy and Infectious Diseases clinician, and Dr. Alexis Shelokov, virologist, recovered Coxsackie virus, group B, type 3, at various times from blood, urine, cerebrospinal fluid and swabs of throat and anus of a 40-year-old patient observed at the Clinical Center. They correlated these isolations with the clinical course of the patient, an Institute scientist. The implications of viruses in urine were discussed in a report on this study in the Journal of the American Medical Association.

The patient had intermittent fever with temperature elevation of 102-104°F. over a 9-day period, with chills, sweating, headache and moderate muscle pain. Since he had been working with viruses, tissue cultures were made from specimens. Coxsackie B virus of a type he had been studying in the laboratory (no accident recollected) appeared in a urine specimen collected on the fourth day of illness. The same virus was also found at various times in blood, cerebrospinal fluid, and pharyngeal and anal swabbings.

The investigators theorize that the virus in urine may have "spilled over" from blood--but they note that no virus could be detected in blood collected approximately 5 minutes prior to collection of the positive urine specimen. Another possibility is that virus was present in blood but masked by antibodies. A third possibility: urine is the concentration of blood factors a thousandfold, perhaps thus increasing the number of virus particles to a detectable level.

The most challenging explanation, however, is that virus was propagating in the cells of the urinary tract, especially those of the kidney. The evidence that monkey and human kidney cell tissue cultures are excellent mediums for the growth of many viruses supports this possibility. In addition, monkey kidneys obtained for tissue culture are occasionally infected or contaminated by (simian) viruses.

Microscopic lesions have been seen at autopsy in kidneys of people with various virus diseases, including poliomyelitis, influenza, mumps, measles, infectious hepatitis, chickenpox (varicella), epidemic (St. Louis type) encephalitis, yellow fever, and cytomegalic inclusion disease.

Abnormal results of urinalyses and other tests of renal function during clinical illness have further suggested to clinicians that the kidney may be involved in a number of viral infections.

In the study just reported, Utz and Shelokov noted a delay in the urinary excretion of phenolsulfonphthalein. Prior to this

abnormal excretion there had been no history of acute or chronic renal disease. The cumulative total given off 2 hours after the injection of the dye was 48 and 50 percent during two tests a week apart--well below normal. About one month later, however, the cumulative excretion of dye was within the range of minimal values in repeated tests. Viruria is suggested as a cause of altered phenolsulfonphthalein output.

Viruria in man was first reported by Jonesco in 1927 in rabbies infection. In 1938 Lepine and Sautter reported the presence of lymphocytic choriomeningitis virus in urine. Little attention was paid to the significance of these and subsequent isolations.

However, Dr. Utz and his associates of NIAID's Laboratory of Clinical Investigation reported in 1957 in the New England Journal of Medicine on the first isolation of mumps virus from urine during intensive studies of 21 patients with apparent mumps infection. They commented that the presence of gross amounts of virus in the urine, although blood specimens failed to yield virus, raised the possibility of the growth of virus in the kidney substance and of kidney damage in mumps.

Utz and Shelokov suggest that the hypothesis of renal virus disease deserves further investigation.

VIRUS OF WESTERN EQUINE ENCEPHALOMYELITIS SURVIVES WINTER IN MOSQUITOES

Dr. W. C. Reeves and associates of the University of California, Berkeley, in a three-year study of mosquitoes in a highly endemic area for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), demonstrated the presence of WEE virus in Culex tarsalis in all months except December. This study was supported by a grant from the National Institute of Allergy and Infectious Diseases.

Western equine encephalomyelitis and St. Louis encephalitis are acute inflammatory diseases of the brain, spinal cord, and meninges caused by two different viruses. Mosquitoes (particularly Culex tarsalis) are considered the chief vectors of these diseases, but whether the reservoirs of these viruses are in mosquitoes or other arthropod vectors, or in vertebrate hosts, such as birds, is not known.

The detected seasonal occurrence of WEE and SLE viruses in C. tarsalis was markedly different. WEE virus was isolated from these mosquitoes during all months except December, which is a minimal blood feeding period. The peak incidence of WEE was from May to July, while SLE predominated in August and September. In only one specimen from a "winter" month, March, was SLE virus

detected. This paucity of isolations, the investigators suggest, may be due to the lower pathogenicity of SLE virus, compared with WEE, in laboratory animals or to the overwintering of this virus in a different way.

Tests provided ample evidence that the winter strains of WEE virus were not capable of the same disease-producing effect in mice and embryonated eggs as the summer strains, and did not elicit as much antibody response in chickens.

The presence of virus in C. tarsalis was found to correspond closely with the seasons of blood feeding by this vector. This, the investigators report, supports the hypothesis that a vertebrate host may have a WEE infection lasting a number of months, making the virus available to the vector when it takes blood from such a host. Birds are probably the host, since 98 percent of the engorged specimens collected from January to May contained nucleated red blood cells.

In summarizing their field study, the California group cautions that ". . . despite the abundance of winter isolations from C. tarsalis it would be premature to conclude that overwintering females of the species are the proven reservoir of infection . . . The possibility that a vertebrate host is the immediate source of wintertime vector infection is as likely as the alternative that virus survives solely in the vector in winter."

HIGHLY PURIFIED VIRUSES OBTAINED IN QUANTITY BY NEW CHROMATOGRAPHY METHOD

A new virus purification technique has been demonstrated by scientists of the National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana, working with investigators of the Carnegie Institution of Washington, Washington, D. C., and Yale University. They showed that column chromatography with ion-exchange chemicals, as employed in the purification of proteins, is applicable to separating viruses and other very small microbes from adherent contaminating materials. A marked degree of purification was achieved even with polioviruses, which rank among the smallest of all viruses.

In column chromatography, the particulate material is washed down a column, such as a glass tube, containing adsorbing chemicals. The different particles have various affinities for the chemicals in the column and flow out at different rates, thus being separated.

The method was used for purification of poliomyelitis (Mahoney, type 1 and Saukett, type 3), vaccinia, ECHO (type 13) and Coxsackie (type A9) viruses from concentrates of monkey kidney

tissue-culture fluids in which the viruses had been grown. Concentrates of Q fever and epidemic typhus fever rickettsiae (microbes larger than viruses but smaller than bacteria) from chick embryo yolk sacs, and of Colorado tick fever virus in brain homogenate from infected mice were also examined.

Visualization of the purified microbes with the electron microscope verified the success of the new method. For example, the rickettsiae of Q fever, a disease of livestock transmissible to man, were revealed free of extraneous matter at a magnification of about 88,000 times. They appeared as well-defined particles consisting of outer cell walls enclosing fluffy, light protoplasmic material.

Some viruses were prepared from tissue cultures grown in the presence of radioactive phosphorus (P^{32}) and correspondence between virus activity and radioactivity indicated that even very small animal viruses can be labeled in this manner.

The investigators are Bill H. Hoyer, Daniel B. Ritter, Richard A. Ormsbee, and Carl L. Larson of the Hamilton, Montana Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases; Ellis T. Bolton, Carenegie Institution; and George LeBouvier, a research fellow of Yale University.

NEWCASTLE VIRUS INFECTIONS IN CATS STUDIED FOR CLUES TO HUMAN ENCEPHALITIS

Newcastle disease is an epizootic virus infection of chicken flocks throughout the world, characterized by viremia with respiratory, gastrointestinal and central nervous system involvement. Animals such as hamsters, mice and monkeys have been experimentally infected, with resulting encephalitis. Drs. Charles N. Luttrell and Frederik B. Bang of Johns Hopkins successfully produced Newcastle disease virus infections in cats. These grantees of the National Institute of Allergy and Infectious Diseases are seeking data relevant in interpreting analogous human disorders. They selected cats as experimental animals because a wealth of neurophysiological information is available and these mammals lend themselves better than do others to detailed clinical follow-up, neuropathological correlation, and physiological study.

The virulent CG-strain of Newcastle virus, chick-adapted, was employed. Among 107 kittens and adult cats exposed, both age groups generally developed encephalitis after direct intracerebral, intracerebellar, or intraspinal inoculation. Dilutions of stock virus out to 10^{-4} logs were effective by these routes. Intranasal or intraocular infections were obtained only in kittens, and only with undiluted or 10^{-1} log dilutions.

Head tilt to one side or the other was generally the first sign of central nervous system involvement, succeeded in a few hours by onset of myoclonus--alternating stiffness and spasm--of head, neck, trunk, and extremities. This could most readily be correlated with lesions either of brain stem or of the spinal cord. The syndrome of myoclonus and limb paresis, and the general anatomical distribution of lesions due to Newcastle disease virus in cats, are similar, suggest Drs. Luttrell and Bang, to von Economo's "hyperkinetic-poliomyelitic form" of encephalitis lethargica in man. Should this disease recur in endemic or epidemic proportions, it might be advisable, the grantees further suggest, to search carefully among members of the myxovirus group for causative organisms.

In their studies there was clear evidence in intranasally inoculated animals of extension along olfactory pathways and their secondary centers to deeper structures of the thalamus and hypothalamus. Thus myxoviruses (mucus associated) are not excluded from pathway to the central nervous system. Apparently, in the present study of Newcastle disease virus infection, the primary pathways, at least, were neuronal in nature, rather than the spread being by contiguous diffusion, and to some extent the agent was disseminated along neurons within functional units. Publication of this work in A.M.A. Archives of Neurology and Psychiatry includes several pages of pathology diagrams and photographs of serial sections of brain and spinal cords from infected and control animals.

CHRONICITY, HIGH PREVALENCE OF SGV VIRUS INFECTIONS CONFIRMED IN NURSERY STUDIES

Salivary gland virus (SGV) infection is one of the more unique viral infections of man. The chronicity of the infection, its high prevalence, and the ability of the virus to involve many tissues, make it probable that SGV is responsible for more clinical disease than is generally recognized.

Seeking to elucidate the epidemiology and clinical importance of human salivary gland virus infections, the National Institute of Allergy and Infectious Diseases has made longitudinal and cross-sectional surveys of virus excretion and serum antibodies in various population groups. A simple technique for detection of SGV in the mouth and urine has been devised, using specific cytopathogenic changes in tissue culture as an indicator.

The NIAID scientists studied salivary gland infection in 108 newborn infants; 47 children aged 8 months to 4 years in a D. C. Department of Welfare nursery; and 26 adult personnel of NIH. All subjects were apparently healthy.

Salivary gland virus was detected in mouth swabs and urine of up to ten percent of the nursery children, and for prolonged periods. No virus was recovered from newborn infants or adults. The virus was recovered only from children who had shown complement fixing antibody to SGV and was never isolated from any of the more than 50 percent who had no demonstrable titer. Studies of several virus-positive children at the Clinical Center showed some metabolic abnormalities, but these were not definitely attributable to the virus infection.

The detection of virus in mouth swabs for periods of 2 to 5 months, and as long as 8 to 24 months after antibody was known to be present, substantiates the chronicity of the infection of the salivary glands. Detection of virus in mouth and urine many months after CF antibody was known to be present suggests that the infection was present over the entire period, since the existence of multiple serotypes of SGV which might reinfect has not thus far been indicated.

Since the virus-positive children possessed specific antibodies on admission, it is probable that recovery of the virus was indicative of experience in the general population, and that at least 10 percent of the children in this age and socioeconomic group are shedding salivary gland virus. This figure is in agreement with frequency of characteristic inclusions in the cells of salivary glands of young children dying of all causes.

The studies provide laboratory confirmation of the chronicity and high prevalence of subclinical salivary gland infection and suggest that persistent urinary tract infection occurs with nearly equal frequency. The data being assembled will be pertinent to future studies to assess the full potential of SGV in disease. For example, the virus is known to affect the central nervous system in cases of fetal or neonatal SGV infection. It seems probable that some CNS damage in childhood may result from unrecognized infections by the virus.

SPONTANEOUS INFECTION OF WILD MAMMAL WITH COLORADO TICK FEVER VIRUS SEEN FIRST TIME

Scientists of the National Institute of Allergy and Infectious Diseases report the first isolation of Colorado tick fever virus from a wild mammal. Drs. Carl M. Eklund, Glen M. Kohls and William L. Jellison of the Institute's Rocky Mountain Laboratory report that hosts were the golden mantled ground squirrel and the porcupine found in western Colorado.

When several members of a religious order in Colorado contracted this disease, the scientists studied the grounds about the monastery and found that golden mantled ground squirrels occurred

abundantly in pastures and roads nearby. They shot 20, captured 4; and collected 2 porcupine, a deer mouse, a chipmunk, and a woodchuck as well.

The investigators took blood samples and removed adult and immature ticks from the animals. When they injected samples from one of the squirrels into test mice, four out of six died. Blood from one of the other squirrels and that from the porcupine killed a mouse in each litter. The investigators identified Colorado tick fever virus by neutralization test in all three cases.

Later blood samples from the four living ground squirrels revealed virus in the blood of two of the animals.

The investigators then infested the four living squirrels with virus-free D. andersoni nymphs. Later, when tested as adults, they found that ticks from one of the squirrels were infected. Colorado tick fever virus had previously been isolated from this same animal.

In a further check, the investigators found that serum samples from one of the squirrels, the woodchuck and the porcupine neutralized Colorado tick fever virus.

POPULATIONS IN PANAMA TESTED FOR INCIDENCE OF DENGUE

Dr. Leon Rosen of the National Institute of Allergy and Infectious Diseases, in collaboration with the Gorgas Memorial Laboratory, conducted a study on the epidemiology of dengue in Panama, a suspected endemic area. Outbreaks of this arthropod-borne virus disease had occurred there in 1904, 1912, and a dengue-like fever epidemic in 1941-42. Hemagglutination-inhibition and neutralization tests employed in this investigation demonstrated the absence of dengue neutralizing antibodies from sera of persons born after 1942, their relatively low prevalence in older persons living in forested areas of the country, and their absence from monkeys known to be susceptible to this infection. This finding, Dr. Rosen reports, demonstrates that dengue was not endemic in Panama in 1954 and it had not occurred there since 1942.

Data obtained on the prevalence by age group of dengue 1 and 2 HI and NT antibodies in Panama City suggest, Dr. Rosen says, that the epidemic of a dengue-like disease in 1941-42 was due to the type 2 virus. This conclusion is supported by the finding of a relatively high prevalence of dengue 2 NT antibodies in the sera of persons born shortly before the outbreak and a complete absence of this type of antibody in the sera of persons born afterwards. This is the first outbreak of this infection known

to be due to type 2 virus--other epidemics in Hawaii, Japan, South Africa, Malaya, and the Society Islands have been attributed to the type 1 virus.

Another group B arthropod-borne virus appears to have occurred in these areas, the investigator says, since a number of persons born after 1942, who lived in forested areas of Panama, were found to have hemagglutination-inhibition but not neutralizing antibodies against dengue and yellow fever viruses. This could have been the result of infection with Ilheus virus which has been isolated in Guatemala, Honduras, Trinidad, and Brazil; St. Louis encephalitis virus which has been isolated in Trinidad; or a presently unrecognized group B virus.

The data presently available on the epidemiology of dengue in the Western Hemisphere suggests that Aedes aegypti is the only species of mosquito responsible for the transmission of the disease in this area. If this hypothesis is correct, dengue should be eliminated from this hemisphere upon completion of the current Aedes aegypti eradication campaign.

CELL BIOLOGY

MECHANISMS OF CELL GROWTH AND VIRUS PRODUCTION REVEALED IN TISSUE CULTURE NUTRITION

Four possible explanations for the requirement of five more amino acids by mammalian cells in tissue culture than those essential for man were presented by Dr. Harry Eagle of the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases. These explanations were suggested as a result of his continuing investigation of the nutritional requirements of cell cultures.

One possibility, Dr. Eagle reported, is that the enzymatic mechanisms which permit the biosynthesis of the five amino acids may have been lost through hundreds of generations of rapid growth in the test tube. Another is that the cells have a limited capacity for biosynthesis, which is sufficient for maintenance and nitrogen balance but not adequate for the rapid growth characteristic of cell cultures. The third possibility is that cultured cells retain the capacity to synthesize these five amino acids but it is not evidenced for lack of appropriate precursors or co-factors. And the fourth is that these five amino acids may also be needed by most body cells but may be supplied either by the action of the intestinal flora on the other amino acids or by the metabolic action of, for example, the liver producing enough of the amino acids to supply the body's needs for nitrogen balance.

Examination of monkey kidney cells in first culture passage within 48 hours after their isolation from the animal host revealed that they needed at least 12 of the 13 amino acids required by long-term cell cultures and it would therefore appear that the biosynthetic mechanisms are not lost through rapid growth in the test tube.

The limited capacity of the cell cultures was observed by Dr. Eagle and his associates in the biosynthesis of glutamine. Although these cells could synthesize glutamine from glutamic acid and ammonia, the amount was not sufficient for growth.

In the biosynthesis of cystine, it was found that these cells could not convert L-methionine into cystine, as the whole animal does, but a number of inorganic sulfur compounds could be used for the synthesis of that amino acid in cultures and would sustain the growth of the cells in a cystine-free medium.

Preliminary evidence indicates, Dr. Eagle said, that the primary function of all but one (glutamine) of the essential amino acids in cell cultures appears to be their utilization for direct incorporation into protein. Glutamine is actively metabolized. Over and above its direct utilization for protein synthesis, it is converted to glutamic acid, aspartic, asparagine, and proline and to a limited degree, glycine, serine, and alanine; and it is also heavily used for the synthesis of the genetic material of the cell, the nucleic acids.

For poliovirus synthesis, recent experiments in Dr. Eagle's laboratory revealed that cells require the presence of a full complement of amino acids within the cell. A vitamin B_6 deficiency caused a striking reduction in the capacity of the cells to make poliovirus and this deficiency resulted in the depletion of such amino acids as glycine, serine, alanine, and proline in the cell pool. The capacity of the cells to produce virus was completely restored upon addition of these amino acids, in the continuing absence of this added vitamin.

These studies of the nutritional requirements and metabolic activities of mammalian cells are expected to provide a better understanding of the mechanisms responsible for cell growth, virus propagation, antibody production and many other complex problems of infectious diseases.

BACTERIA

105 CYSTIC FIBROSIS PATIENTS OBSERVED FOR DISEASE SYMPTOMS AND TREATMENT EFFECTIVENESS

Drs. Harry Shwachman and Lucas L. Kulczycki of the Children's Medical Center, Boston, say "The widely held belief that cystic fibrosis of the pancreas is inevitably a fatal disease in

infancy or early childhood is not substantiated by this study." Ninety-five of the 105 patients followed from 5 to 14 years from the time the diagnosis of cystic fibrosis was established are still living. The survival beyond childhood of patients with this disease is explained by improved diagnostic methods, with the early recognition of mild cases, and by the application of effective therapeutic measures.

In selecting the patients for this study, the Boston investigators included only those who had been under observation for a minimal period of 5 years. The diagnosis of cystic fibrosis was established by examination of duodenal fluid, the sweat test beginning in July 1954, and the finger imprint method for chloride after August 1956.

The patients were divided arbitrarily into seven groups according to age at which diagnosis of cystic fibrosis was made. Ten patients in one group had meconium ileus--their cases were diagnosed within the first few weeks of life--and, with one exception, they were relieved of their obstruction surgically. In 21 patients, diagnosis was made at less than 6 months of age; in 15 between 6 months and 1 year; in 18 between 1 and 2 years; in 32 between 2 and 8 years; and in 9 between 8 and 16 years.

The general principles of therapy employed in this study, the investigators say, were more or less uniform with emphasis on individualization. Prior to September 1948, sulfonamides, penicillin, and streptomycin were the only agents available. The beneficial effect of aureomycin was noted in September 1948, and it has proved to be one of the most effective antibiotic agents used in the treatment of these patients. Terramycin, introduced early in 1950, has been equally effective. Tetracycline hydrochloride, which appears to be less effective than aureomycin and terramycin, has been used in milder cases or under special circumstances. Chloramphenicol and erythromycin have been used liberally during the past 3 years, and novobiocin, only sparingly during the past year. Neomycin administration by aerosol has been employed almost as frequently as penicillin and streptomycin aerosols during the last 2 years. The treatment plan has improved further with the addition of spraying and wetting agents.

Eighty-seven of the patients received antibiotics daily, 14 were given them intermittently, and 4 only when having symptoms of respiratory infection, which was rather infrequent. Aureomycin was the most frequently used single antibiotic--the dosage varied from 10 to 25 milligrams per kilogram per day--and was given for the longest period of time, i.e., more than 8 years in 10 patients. Terramycin was used singly and was alternated with aureomycin in the same dosage schedule. Inhalation therapy has not been assessed for its effectiveness because it was introduced too

recently to influence the results of the study.

The treatment aimed at improving nutrition consisted of a liberal diet in which a relatively high protein intake was emphasized, and an effort was made to reduce the total fat intake. In practically all patients with pancreatic insufficiency, substitution therapy with pancreatin (Viokase) was given with each meal. Water miscible multivitamin preparations were given in twice the usual dosage and vitamin B complex was used liberally.

The clinical evaluation of these patients to determine the severity of the disease, effect of therapy, and to compare one patient with the next is based upon four criteria: 1) general activity, 2) physical examination, 3) nutritional status, and 4) findings on roentgenograms of the chest. Each category is given equal weight in the scoring system.

Ten of the 105 patients died--none of these were originally in the excellent or good category. The present condition of the remaining 95, compared with their original status is: 47 are better; 27 are the same; and 21 are worse. Forty-one of these patients are over 10 years of age and the eldest is 33 years old and the father of three children. During the last 5-year period of the study, no patient from the excellent and good groups ended up in the severe category; on the other hand, three patients having severe disease initially are now in the mild group.

Their study reveals, Drs. Shwachman and Kulczycki say, that the effect of pulmonary involvement reflects the severity of cystic fibrosis, inasmuch as the cause of death is eventually pulmonary insufficiency from a combination of diffuse obstructive phenomena and infection. The investigators believe their results indicate the effectiveness of the medical regimen employed and emphasize the value of pursuing a vigorous therapeutic program in a disease which is still so little understood.

RECTAL PROLAPSE FOUND TO BE COMMON COMPLICATION IN CYSTIC FIBROSIS

In another clinical study conducted by Drs. Lucas L. Kulczycki and Harry Shwachman at the Children's Medical Center in Boston, rectal prolapse (abnormal descent of the mucous membrane of the rectum) occurred in 22.6 percent of 386 patients with cystic fibrosis. This complication was most frequent in children between 6 months and 3 years of age, and was rarely encountered in patients after the age of 5 years.

In 16 patients, rectal prolapse was the initial complaint of cystic fibrosis; and in 3, the diagnosis of cystic fibrosis was made 7, 8, and 10 years after this initial complaint.

This complication may occur as an isolated event or may be recurrent, extending over a 5-year period. In 48 patients this symptom lasted longer than 6 months.

Dietary therapy (primarily a high-protein and a reduced fat intake) combined with pancreatic replacement and antibiotics to control the pulmonary infection, the investigators said, will usually correct many of the factors responsible for rectal prolapse. Surgical correction in their experience was rarely, if ever, indicated.

The realization that such a complication commonly occurs in cystic fibrosis, Drs. Kulczycki and Shwachman stated, should suggest a more careful search for other manifestations of this disease in infants and children with rectal prolapse of unknown etiology, and thereby permit early diagnosis and treatment.

METABOLIC CHANGES PRODUCED IN MAN BY ANTIBIOTIC THERAPY

Dr. Maxwell Finland of Boston City Hospital, in collaboration with a group of Harvard Medical School researchers, reported the results of a 3-year nutritional study of undernourished men given relatively large oral doses of tetracycline drugs (aureomycin, terramycin) and polypeptide antibiotics (bacitracin plus polymyxin). The metabolic effects observed in the patients on tetracycline therapy were losses of body weight, increased urinary excretion of riboflavin and nitrogen, increased concentration of nonprotein nitrogen in the serum, and resultant negative or reduced positive nitrogen balance. The polypeptide antibiotics produced marked changes in fecal flora but no metabolic alterations. Dr. Finland is a grantee of the National Institute of Allergy and Infectious Diseases.

The bacterial flora changed in all the patients during administration of the antibiotics but differed considerably in each patient.

The seven men selected for this study gave prior histories of inadequate dietary intakes with accompanying weight loss and displayed physical evidence of undernutrition; this condition was confirmed by gains in body weight and positive nitrogen balances upon provision of adequate intakes of food. They were given a constant diet of at least 75 gm. of protein and 2700 calories daily, were maintained on a metabolic ward, and were ambulatory.

The antibiotics used in this study were given four times a day in oral daily doses of from 20 mg. to 3.0 gm. of aureomycin, 2.5 gm. of terramycin, and 100,000 or 150,000 units of bacitracin

and 500 mg. of polymyxin B. sulfate. Patients were observed for 9 days prior to, during, and after therapy.

Fecal excretions of vitamins were not altered in any consistent direction during the antibiotic therapy but increases in the urinary excretion of three amino acids--tryptophane, histidine, and threonine--were associated with aureomycin administration. The magnitude of increases, however, was not great enough to be of nutritional significance.

The character of the stools changed during administration of the antibiotics, becoming heavier and bulkier and containing an increased amount of undigested food particles and fibrous material. These changes, the investigators report, suggest that the antibiotics may interfere with digestive processes of the intestinal tract in some way not related to action of bacteria.

The bacterial organisms most frequently found in the feces were coliforms (Escherichia coli and Aerobacter aerogenes), enterococci, Bacillus subtilis, and clostridia (Clostridium welchii). During or after treatment, those most frequently appearing were hemolytic Micrococcus pyogenes var. aureus (coagulase-negative), Proteus, and yeasts (Candida albicans). Clostridia were markedly decreased in number or entirely absent in five of the seven patients during antibiotic therapy. Organisms that first appeared in the stools after antibiotic therapy started were usually markedly resistant to the drug that was given.

In summarizing their study, the researchers suggest that the tetracycline compounds may exert their metabolic effects directly on the tissues rather than indirectly through their influence on the normal bacterial flora of man. They also point out that since there is a close relationship between riboflavin and the metabolism of tissue protein, it is possible that the increases in urinary riboflavin excretion are related to the increases in urinary excretion of nitrogen.

UNIQUE AREAS OF VALUE SHOWN IN TESTS OF TWO NEW ANTIMICROBIAL DRUGS

New observations on blood and urine levels of sulfamethoxypyridazine and comparison with its relative, sulfachloropyridazine, serve to point up their contrasting areas of usefulness.

Wilfred F. Jones and Maxwell Finland of the Thorndike Memorial Laboratory studied the action of these drugs on patients at the Boston City Hospital. The investigators are grantees of the National Institute of Allergy and Infectious Diseases. Animal work on the methoxy derivative by other investigators, had indicated its promise as an antibacterial agent. In one study it proved generally superior to all the popular sulfonamides except sulfadiazine.

In the patient studies, Jones and Finland found the methoxy derivative has a plasma half-life five times as long as its related compound. In addition, patients took seven times as long to excrete one-half an oral dose of the sulfamethoxypyridazine.

Each of the drugs showed comparative levels of concentration in the blood after a dose but the methoxy compound maintained a nearly constant level between doses--not true of the chloro derivative. This latter compound, however, did display a higher concentration in the urine.

The investigators conclude that sulfamethoxypyridazine may profitably be used where low and infrequent doses are necessary in lengthy treatment of various subacute and chronic infections.

They point out that this type of dosage should be more economical as well as useful for a prolonged suppression of those difficult to erase, chronic infections.

Sulfachloropyridazine, on the other hand, is excreted quite rapidly. Its high urine concentration suggests that it may be promising in the treatment of urinary tract infections. The grantees warn, however, that the large doses needed for maintaining correct blood levels might lead to crystalluria followed by irritation of the urinary tract.

EFFICACY OF TULAREMIA VACCINE IN MICE IMPROVED BY ADDING WHOOPING COUGH VACCINE

Dr. Lolita Pannell of the Medical College of South Carolina in Charleston discovered two new methods of improving tularemia vaccine. One incorporates whooping cough vaccine as an adjuvant with tularemia vaccine, and the other combines whooping cough vaccine and a supposedly antigenic material elaborated by the tularemia organism during growth. This work is supported by a grant from the National Institute of Allergy and Infectious Diseases.

While using Hemophilus pertussis sensitized mice to demonstrate tularemia strain differences, Dr. Pannell discovered that whooping cough vaccine alters the susceptibility and resistance of mice to tularemia. Pretreatment with this vaccine increases the resistance of mice when challenged with a moderately virulent strain of tularemia. On the other hand, simultaneous inoculation of the vaccine and the living tularemia organism makes the mice more susceptible to the infection.

The finding that whooping cough vaccine could increase protection against tularemia led the investigator to compare the efficacy of the current tularemia vaccine singly and in combination with

H. pertussis. The addition of whooping cough vaccine increases the agglutination titer two- to four-fold over that of the tularemia vaccine alone against low challenge doses of tularemia.

During the course of a study on the toxicity of Bacterium tularense, Dr. Pannell observed that inoculation of filtrates into white mice confers a certain degree of protection against subsequent challenge. Tests of the broth filtrates show that they contain a substance which is elaborated into the medium during growth and which is as immunogenic as intact cells or extracts thereof. The immunogenicity of the filtrates is as good as the current tularemia vaccine and is improved by the addition of whooping cough vaccine.

The final proof of the value of incorporating whooping cough vaccine into a good protective tularemia vaccine, Dr. Pannell says, must await the production of a better Bacterium tularense preparation. She also suggests that a study of the antigenic material of the filtrates, its nature and requirements for optimal elaboration, would illuminate the mechanism of immunity in tularemia.

M. TUBERCULOSIS CELL WALL MAY PROVIDE FRACTION FOR MORE POTENT VACCINE

Basic investigations of the antigenic components of various bacterial agents have provided new leads for isolation of active protective fractions. Special attention is directed toward the immunologic properties of the tubercle bacillus because of the need for an effective vaccine against tuberculosis. This would be of particular benefit to groups at risk in this country and throughout the world.

At NIAID's Rocky Mountain Laboratory in Hamilton, Montana, Edgar Ribi, Carl L. Larson, Robert List, and William Wicht have employed the disciplines of biophysics and bacteriology to obtain intact, pure cell walls of two strains of Mycobacterium tuberculosis (BCG and H37Ra) and also of M. butyricum, a common organism resembling the tuberculosis agent but relatively nonpathogenic.

In obtaining cell walls of mycobacteria free of extraneous material and protoplasm, the NIAID investigators employed initially the procedure of Salton and Horne (University of Cambridge, England). This involves centrifugation to wash away soluble cell components. The deposit is resuspended and gently shaken with glass beads of about one-eight inch diameter. These crack the cell walls, allowing the protoplasm to be centrifuged.

The Rocky Mountain Laboratory investigators point out, however, that special precautions must be taken for selective disruption

of mycobacteria. Suspensions of detergent-washed cells must be monodispersed when exposed to mechanical vibration and the products of disruption must also be maintained in a dispersed state to allow final separation of cell wall from protoplasm. Electron micrographs reveal that cell walls of mycobacteria obtained were not fragmented, but were merely cracked at one end. There was little or no protoplasmic remnant.

TWO SEPARATE PROCESSES INDICATED IN STAPHYLOCOCCAL RESISTANCE TESTS

Small quantities of chloramphenicol induce an alteration in Staphylococcus aureus which allows it to grow in the presence of this antibiotic.

Dr. H. H. Ramsey of the University of Oklahoma School of Medicine drew this conclusion from laboratory experiments supported by a grant from the National Institute of Allergy and Infectious Diseases.

Medical scientists know little about the mechanisms of antibiotic resistance. Our knowledge of how an antibiotic inhibits and how an organism overcomes this inhibition lags far behind the discovery and use of antibiotics. Staphylococcal resistance is today one of our gravest medical problems, and, since investigators can easily produce, in vitro, strains of many bacteria resistant to all antibiotics, other forms of resistance will probably emerge in the future.

To cope with these difficulties makes it necessary to understand how resistance arises and how it can be prevented.

Dr. Ramsey notes that resistance to antibiotics is usually envisioned as the result of either spontaneous mutation or adaptation. In mutation, the antibiotic acts merely as an agent favoring the growth of mutant organisms appearing at regular intervals regardless of the antibiotic. On the other hand, observes Dr. Ramsey, with adaptation the antibiotic itself induces the change that leads to resistance. Resistance to high concentrations of chloramphenicol is considered to be the result of repeated selection of resistant mutants.

In the following experiment Dr. Ramsey suggests that resistance to low concentrations of chloramphenicol is the result of adaptation.

He inoculated staphylococcus into a broth containing no chloramphenicol and found 6 percent of the staph resistant initially. This grew to over 50 percent and then rapidly decreased. Thus he observed that rapid cellular metabolism and cell division resulted in a certain amount of increased resistance in itself.

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Dr. Ramsey then inoculated staph into tubes containing varying small amounts of chloramphenicol. In these cases the staph increased its resistance to well over 90 percent after several hours and maintained that level of resistance thereafter. Chloramphenicol seems to force the staph to maintain resistance as long as it is present.

In broth containing chloramphenicol, many more cells became resistant in a certain time interval than were produced by cell division. Since resistant organisms usually have a growth rate similar to or slower than the strains from which they are derived; and since prior experiments have shown that resistant forms of M. pyogenes have a growth rate equal to that of sensitive forms in the absence of chloramphenicol, the evidence favors adaptation rather than mutation as the explanation for increased resistance.

It further suggests that adaptive resistance occurs because of the presence of the antibiotic and is more permanent than the nonspecific resistance resulting from cellular metabolism and growth.

GROWTH INHIBITING AGENT FOR TUBERCULOSIS ORGANISM FOUND IN GAMMA GLOBULIN FRACTION

Seeking a key to the direction of the tuberculous process, Dr. Florence B. Seibert, University of Pennsylvania, made a study of the interplay between antibodies and antigens in the immune mechanism. The results of these experiments show that antibodies have no direct bacterial destructive or growth restricting effect on finely dispersed BCG organisms in vitro. Her study also reveals the presence of an effective antimicrobial substance in the gamma globulin fraction, especially from normal serums, which would be independent of antibodies. Dr. Seibert's work is partially supported by a grant from the National Institute of Allergy and Infectious Diseases.

In tests by this investigator of whole normal, immune, or tuberculous serums, fractions of these serums, antigens, combined antiserums and antigens, and lysozyme against the BCG organism, all fractions exhibit an antimicrobial effect, but not to the same degree. A study of the antibodies for their effect on the tubercle bacillus demonstrates that they themselves are probably not responsible for inhibiting its growth but that specific ones, by diverting certain antigens, indirectly aid in the normal process of resistance. An example of this is the antipolysaccharide antibody, which by combining with freely circulating tuberculopolysaccharide diverts it from occupying the effective groups of a naturally occurring antimicrobial agent, thus leaving them free to act on the tubercle bacillus.

The antimicrobial agent for tubercle bacilli found in the gamma globulin fraction was heat stable, and, therefore, not complement. Its effect in serum was counterbalanced to a large extent by albumin, a growth stimulant. The inhibitory effect of this substance on the bacillus, Dr. Seibert says, could be markedly decreased by the presence of free tuberculopolysaccharide, suggesting that the agent acts primarily through its association with the polysaccharide. The presence of the antimicrobial agent in normal as well as immune serum led the investigator to suspect it to be lysozyme. Lysozyme has an inhibitory effect in vitro on the growth of tubercle bacilli similar to gamma globulin, is known to occur in serum, and attacks the polysaccharide component of bacilli.

The importance of the tuberculopolysaccharide in the tuberculous process cannot be overemphasized, Dr. Seibert says, because of its ability to interfere with the immune mechanism. She stresses the need for antipolysaccharide antibodies against such an invader to curb infection and suggests that they could be supplied by passive transfer of immune serum. She says that more of the naturally occurring bacteriostatic or bactericidal agent is also needed to curb the infection.

Experiments are now under way, this investigator says, to induce the production of antibodies by vaccinating animals natively resistant to tuberculosis. Further studies will be made to determine the effectiveness of these antibodies, when combined with a nonspecific bactericidal agent, such as lysozyme.

IMPROVED METHOD PROVIDED FOR IDENTIFYING BACTERIA CAUSING INFANT DIARRHEA

Dr. Cyril S. Stulberg and a group of researchers at The Children's Hospital in Detroit successfully adapted the fluorescent antibody technique to the identification of disease producing types of Escherichia coli, causative agents of infantile diarrheal disease. The advantages of this test, the Michigan group reports, are that it can be performed within 15-25 minutes directly from stool or rectal swab specimens, is simple and practical, and is more specific and sensitive than identification by culture and specific agglutination. This work is supported by the National Institute of Allergy and Infectious Diseases.

Stored frozen material from an epidemic in 1954 of diarrhea caused by intestinal disease-producing E. coli extensively studied by conventional methods, permitted a thorough evaluation of the specificity and sensitivity of the fluorescent antibody method. The readings of results obtained by this technique were recorded without knowledge of the results obtained on the comparable stool specimens and swabs in 1954 or of the results of the standard bacteriologic methods used on duplicate specimens.

All of the stool specimens corresponding to the rectal swab cultures found positive in 1954 for E. coli gave positive results with the fluorescent antibody technique. In addition, 39 positive results were obtained by this method from the remaining 61 specimens from cases originally reported as negative by rectal swab culture. In each of these cases, the infant had at one time received neomycin therapy, and enteropathogenic E. coli organisms had been identified in the stool, either previously or subsequently, by the conventional methods.

The sensitivity of the fluorescent antibody technique enables it to identify organisms that have lost the power of reproduction (presumably as a result of antibiotic therapy) or are present in such small numbers that they escape detection in the mixed stool flora. Positive findings could be obtained by this method long before there was sufficient growth to permit identification by the conventional methods. One additional advantage of this method is that it offers a convenient and rapid technique for serial follow-up studies on patients excreting organisms.

The investigators report that they are preparing a polyvalent antiserum for the identification of 10 different known intestinal disease-producing strains of E. coli from diarrheal stool specimens.

PRE-INCUBATION ENHANCES ANTIBACTERIAL PROPERTIES OF BANK BLOOD

"Many investigators have concluded that a certain small percentage of bank bloods will become contaminated with bacteria during collection in spite of the most rigid safeguards and precautions taken," comment NIAID grantee Philip Geller and associates, University of California. Seeking a practical means of reducing contamination, they observed that pre-incubation at 37°C.--the optimum temperature for enhancing antibacterial action of antibodies and complement--resulted in a reduction of many but not all types of bacteria in whole fresh blood subsequently stored at bank refrigeration temperature of 4°C.

Many types and strains of bacteria and numbers of blood samples were employed in the experiments. With the warm-growing bacteria, the investigators conclude that brief incubation of blood at 37°C. probably accelerated the rate of killing by antibacterial factors present and the survivors, unable to multiply at 4°C., eventually died out. These same factors may have been equally active at 37°C. against the cold-growing bacteria, but the survivors in this instance were not suppressed by low temperatures and in time multiplied to high levels.

Relatively small numbers of many strains of cold-growing Pseudomonas, Achromobacter, Paracolobactrum, Aerobacter, and Escherichia were able to multiply rapidly enough in pre-incubated

and refrigerated blood to reach potentially dangerous concentrations during the 21-day period of observation. This indicates that constant storage of bank blood at 4°C. is not in itself an adequate means of inhibiting growth or of insuring sterility of the blood. This is in accordance with reports that these same types of cold-growing organisms are most often implicated in serious or fatal transfusion reactions. The investigators believe that it is inevitable that additional blood reactions resulting from contamination will continue to occur until some effective means, perhaps the addition of antimicrobial substances to blood, are found to suppress the growth of all types of contaminants. They observe that recent investigations with antibiotics appear promising, but additional studies are necessary to determine whether such agents can be added to bank blood safely and effectively. Use of various antiseptic agents and dyes to prevent bacterial contamination has been generally unsatisfactory.

PARASITIC DISEASES

ROUNDWORM INFECTIONS EFFECTIVELY TREATED WITH DITHIAZANINE

Dr. J. C. Swartzwelder of the Louisiana State University School of Medicine, and his associates, reported that dithiazanine eliminated strongyloidiasis (parasitic worm infection) in 89 percent of the patients treated with this drug. These studies are supported by the National Institute of Allergy and Infectious Diseases.

Comparing dithiazanine with a large number of compounds tested for their activity against strongyloidiasis, the investigator considers this drug the most effective treatment for this infection.

Eighteen male patients with roundworm infections were each given 200 milligrams of dithiazanine three times daily for 5 days. The treatment period was extended to 21 days when it became obvious that the shorter term did not provide adequate therapy in all cases. The drug was administered in the form of syrup and special enteric-coated tablets designed to delay release of the compound until after passage through the stomach.

In 16 of the 18 patients given the complete course of treatment, diagnostic forms of the parasite were not demonstrable in stools and duodenal drainage fluids. In one of the two cases considered treatment failures, the drug was discontinued after 6 days because of the patient's psychiatric status, which was unrelated to therapy. Thus, only one of the 17 patients who received the medication for 21 days had evidence of parasitic infestation after treatment.

Two patients had mild and transient diarrhea, without cramping, and one patient had two episodes of vomiting during the 21-day course of therapy. The results of urinalyses, hemograms, blood urea nitrogen determinations, and liver function studies revealed no significant change in any of the patients during and after therapy.

In addition to the patient study, an evaluation was made of the worm destructive activity of dithiazanine against murine strongyloidiasis by experimentally infecting rats with Strongyloides ratti, treating them with the drug, and performing autopsies to determine whether or not infection persisted in the intestine after chemotherapy.

Infection was completely eliminated in the 17 rats treated with dithiazanine. No adults, eggs, or larvae of S. ratti could be found in the autolyzed mucosal scrapings of the animals after therapy. Examination of the intestines of the treated rats showed the intestine to be discolored by the dye released by the drug.

In an earlier study supported by this Institute, Dr. Swartzwelder found dithiazanine effective in the treatment of patients with either single or multiple intestinal helminth infections such as whipworm, threadworm, roundworm, and pinworm.

MORE EFFICIENT ERADICATION OF DISEASE CARRIERS SOUGHT IN ANIMAL SYSTEMIC INSECTICIDE

Dr. B. Wayne Arthur and associates of the Department of Zoology and Entomology, Alabama Polytechnic Institute, Auburn, have received grant support from the National Institute of Allergy and Infectious Diseases during the past few years in studying problems concerning disease-spreading, blood-sucking ectoparasites. These include the stable fly, the Gulf-Coast tick, the bed bug, the lone star tick, and the yellow fever mosquito.

The NIAID grantees conducted a number of experiments on potentially effective systemic livestock insecticides. Conventional methods of spraying, dusting and dipping with various poisons are generally time-consuming and expensive. The ideal systemic insecticide would be put into livestock feed, absorbed into the animal's bloodstream with toxic effect only to the parasites feeding on that blood.

Among several promising compounds, Dow ET-57 proved effective against the common cattle grub when administered in the food as a large pill.

Although it is not regarded as the ultimate in control of the parasite, this systemic demonstrates the practicability of the approach. It circulates with the body fluids and destroys grubs that have burrowed into the flesh--first systemic proved capable of doing so.

ET-57 used in control of grubs gives an extra bonus: partial elimination of some species of roundworms. This antihelmintic effect was revealed in tests by the U. S. Department of Agriculture at the Regional Animal Disease Research Laboratory in Auburn. For example, ET-57 equaled phenothiazine in removing an average of 98 percent of the stomach worm Haemonchus placei, and got rid of 59 percent of the small-intestine worm Cooperia punctata.

The systemic is being marketed for limited use against grubs in certain areas of Iowa, Nebraska, South Dakota, and Wyoming. Treatments are not made later than 60 days before slaughter and lactating cows are not treated because the possibility of residues toxic to humans has not been studied adequately. This is one of the facets of systemic insecticides that the NIAID grantees will continue to investigate.

A NEW DRUG SHOWN TO BE EFFECTIVE AGAINST HUMAN HOOKWORM INFECTIONS

Bephenium salts showed significant activity against moderate to heavy hookworm infections in mental patients in tests conducted by Drs. Martin D. Young and G. M. Jeffery of the National Institute of Allergy and Infectious Diseases, Epidemiology Section, Columbia, S. C., in collaboration with staff members of the South Carolina State Hospital.

Hookworm (Necator americanus) infections in mental patients constitute a serious problem because of their severity and the difficulty of effective treatment. The drugs in common use against hookworms ordinarily give poor results with such patients. A search for better drugs has been carried on at the Columbia laboratory during the past 8 years. Thirty-five different old and new drugs have been tested for a total of 770 patient trials. Bephenium, the new drug, shows promise of being more effective against this parasitic infection than the commonly used tetrachlorethylene.

In the 100 patient trials of the three salts of bephenium (generic name given to a new group of compounds), all three, in the best regimens, gave better results than tetrachlorethylene, which has been the standard treatment for hookworm infections for a long time. The chloride and hydroxynaphthoate salts, given over a 5-day period in total amounts of 5.28 and 10.5 grams

respectively, resulted in 85 percent reduction of hookworm eggs in patients. The bromide given in the amount of 4.724 grams over the same period produced 67 percent egg reduction. Tetrachlorethylene caused a 58 percent reduction.

The density of the worm eggs in the feces was determined by the Stoll method--two determinations being made immediately before the drug was given and two after the treatment. There were no pretreatment nor post-treatment procedures, such as purges, etc.

Only eight side reactions were experienced as a result of this therapy and they occurred shortly after the drug was given. Vomiting episodes were associated with the larger dosages.

Two other intestinal nematode parasites were also affected by bephenium. Some activity was exerted by the drug against Trichuris, and 7 our of 14 Enterobius infections were cured.

PARASITES IN UTERUS OF RATS WITH CHRONIC TOXOPLASMOSIS CAN CAUSE INFECTION IN OFFSPRING

The finding of latent Toxoplasma parasites in the uteri of rats suggests the possibility of congenital toxoplasmosis in offspring of human mothers with chronic infections, Dr. Jack S. Remington of the National Institute of Allergy and Infectious Diseases reports. Although women with acute toxoplasmosis can transmit it to their offspring, it has not been determined whether mothers with chronic forms can also transmit toxoplasmosis to their children.

This obscure parasitic disease infects at one time or another a high percentage of people in the United States, although most develop immunity. The most common type of acute toxoplasmosis is a mild disease resembling glandular fever. A chronic form of the infection is a blinding eye disease. Other forms involve the unborn or newborn child, causing premature birth, death, or brain damage and blindness.

In experiments designed to provide every opportunity for transmission of the infection to the offspring, Dr. Remington and his associates inoculated 22 female rats with various numbers of organisms and after 6 to 8 weeks, when the infection was chronic, bred these animals. A total of 140 offspring was produced in 22 litters. Of the newborn, three in one litter were found infected with parasites. All of the mother rats had positive dye tests for toxoplasmosis. In another series, tests of the uteri of 24 chronically infected rats showed infection in two. The offspring did not acquire the infection.

Only occasionally is toxoplasmosis transmitted from the chronically infected mother rat. Apparently parasites within

a pseudocyst may be transported to the fetal tissues when the placenta erodes that portion of the uterus containing them. The question of such occurrence in humans might be approached through studies of populations in which there is a high prevalence of Toxoplasma antibodies even before child-bearing age, as in Tahiti.

SCHISTOSOMIASIS SYMPTOMS OF MAN CAN NOW BE REPRODUCED IN LABORATORY ANIMALS

One of the symptoms of schistosomiasis in man, enlarged esophageal veins, can now be reproduced experimentally in a laboratory animal, according to Drs. Kenneth S. Warren and William B. DeWitt of the National Institute of Allergy and Infectious Diseases. This finding, in addition to two other symptoms reproducible experimentally, they explain, will make it possible to study the pathological changes in the animal and relate them to the disease as it exists in humans.

Schistosomiasis, a debilitating parasitic disease which afflicts an estimated 110 million people in other parts of the world, is not a rapid killer but spreads its death slowly by damaging the intestines, bladder, liver, or lungs. This chronic affliction, caused by flukes invading the body and living in the veins of the abdominal cavity, often makes its victims too sick or too weak to work. A fully satisfactory treatment for schistosomiasis has not been found and the present efforts for controlling the disease are based primarily on chemically killing the snails which serve as intermediate hosts to the flukes.

Thirty-seven of 101 Swiss albino mice developed esophageal varices 10-16 weeks following infection with a Puerto Rican strain of Schistosoma mansoni. These varices were never seen in uninfected mice or in mice infected 8 weeks or less. Their size and distribution differed but they were all periesophageal. In most cases there was one large tortuous vessel, but in many there were two. These arose from gastric and splenic veins and occasionally from the hepatic venous plexus. They usually terminated in the inferior vena cava or in the azygos vein. Sometimes they ended in the pulmonary vessels.

The average spleen size of the mice with varices was always greater, but the size of the liver was not different from that of mice without varices.

It was shown in this study that hepatomegaly precedes splenomegaly, which occurs coincident with the onset of esophageal varices. This points out, the investigators said, the relationship of prior liver disease in schistosomiasis to the subsequent development of splenic enlargement and esophageal varices.

STANDARD DRUG STUDY TOOL SEEN IN CONSISTENT INTERACTION OF MOUSE AND T. VAGINALIS STRAINS

A mouse strain that reacts consistently with a strain of Trichomonas vaginalis offers possibilities as a laboratory animal in screening for drugs that might act through the blood stream against the parasite. Variability of T. vaginalis infections has been a particular problem in evaluating therapy in animals or humans.

The finding of an apparently useful combination of host-parasite strains, a preliminary result of a long-term laboratory and clinical study of trichomoniasis, is reported by Lucy V. Reardon and Dr. Leon Jacobs, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases. Trichomoniasis is a common, non-fatal infection of the genito-urinary tract. Its highest incidence is in women between the ages of 30 and 50.

The NIAID scientists studied 11 strains of T. vaginalis injected into various strains of mice. The first tests performed involved a comparison between two strains of the parasite derived from a human case of severe vaginitis (C-1 strain) and from a mild case (R-strain).

In one of the strains of mice (A/LN) the C-1 strain of parasite regularly produced fatal infections. During 10 experiments, 102 of 107 A/LN mice succumbed to infection by C-1 strain T. vaginalis. On the other hand, comparable inocula of the R strain of parasite never produced fatalities. This excellent correlation between a strain's virulence in humans and in mice was not maintained throughout other experiments, although some correlations were observed.

Infection with T. vaginalis in women (in men it is usually symptomless) may be inapparent or may be manifested by a wide range of symptoms and may lead to severe inflammation of the vaginal and surrounding tissues. Clinical observers have reported widely different degrees of inflammation in women with the infection. The research seeks an understanding of the factors involved in this variable response.

The investigators observe that because of the multiple factors involved in susceptibility, the degree of pathology seen in a particular patient must be correlated with the resistance of the individual as well as the virulence of the strain. It may develop, from further study, that a statistical correlation can be obtained between the virulence of strains and the symptoms of patients, but this will require much more data than are presently available. This host-parasite relationship is also important to the understanding of many other infections.

The researchers believe that because the results with the C-1 strain parasite in A/LN mice were quite consistent, the most definite conclusion to be drawn from the study thus far is that it appears feasible to use intraperitoneal infection in mice as a standard tool in drug studies.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

ARTHRITIS AND METABOLIC DISEASES

1958

Items of Interest on Research Studies Conducted and Supported by the

National Institute of Arthritis and Metabolic Diseases

Medical research is delving ever more deeply into the very substance of life, both in health and disease, in search of the keys to the cause and cure of many diseases and disorders.

As this search continues it becomes apparent that the ultimate answers will not come easily, but heartening progress continues to be made. Physicians, working with patients, and scientists in many specialties are making advances every day which contribute toward the health and welfare of people everywhere.

Within the broad area of research covered by the programs of the National Institute of Arthritis and Metabolic Diseases lie many of the most complex problems yet remaining to be solved. These are being attacked by special programs involving a triple threat approach. In its own laboratories and clinics the Institute conducts both basic and clinical research; by means of its grants program it supports research in non-federal institutions throughout the country conducted by leading experts in their special fields; and through the agency of an extensive training grants program it encourages and supports the training of physicians and scientists in the special fields pertinent to its work.

The research advances that are being made are exemplified by the few selected accomplishments which are reported on the following pages.

Prepared, January 1959

RHEUMATIC DISEASES

Research progress against the rheumatic diseases has been highlighted during the past year not only by advances of immediate value to patients, such as new or improved forms of treatment, but also by findings of a more basic nature which contribute to a fuller understanding of the disease processes themselves and will eventually lead to the means for prevention and cure.

With present methods of treatment, including new, safer, and more effective drugs developed in recent years, much can be accomplished, even for bed-ridden arthritics, and the outlook continues to improve. Combined with better rehabilitation measures and with newly improved diagnostic test procedures which enable physicians to begin proper treatment early in the course of the disease, the new drugs make it possible to prevent, in a great majority of cases, the crippling and disability so common in the past as a result of rheumatoid arthritis.

Meanwhile, scientists are probing ever deeply into underlying facts, including the biochemistry of collagen, the jelly-like substance which is a key constituent of connective tissue, the material of which the supporting structure of the body (cartilage, tendons, ligaments) is constructed. This is the material which is attacked and sometimes destroyed by a number of the rheumatic diseases, and about which much more knowledge is required to advance the search for the cause or causes of these diseases.

DEXAMETHASONE, POTENT NEW ARTHRITIS DRUG STUDIED

Dexamethasone, a new synthetic steroid drug 25 times more potent in the treatment of rheumatoid arthritis than hydrocortisone and 6 times more potent than prednisone, has received its first intensive trial in a small number of cases by clinical investigators at the Institute. Simultaneously, the drug received a large-scale clinical trial by Dr. E. W. Boland, Los Angeles, California. The new compound, now available under the trade names Decadron and Deronil, was found to be effective in suppressing the symptoms of rheumatoid arthritis in many patients previously treated unsuccessfully with other antirheumatic drugs.

Dexamethasone appears to be relatively (but not completely) free of major side effects. The production of ulcers, bone fractures, hypertension and mental changes have thus far occurred less frequently with the new drug than with older steroids. Another advantage of dexamethasone is that it appears to cause less interference with carbohydrate metalbolism than the other steroids.

Dexamethasone is another in the ever-growing list of steroids which have been developed since the beneficial effects of cortisone were first discovered in 1948. Although it is the most potent one now available, it is still too early to fully evaluate it as a therapeutic agent. The important question that must still be answered is whether prolonged dexamethasone administration will continue to suppress disease activity effectively and yet not give rise to serious side effects. The new steroid appears to be a definite step forward in the treatment of rheumatoid arthritis but a longer period of evaluation will be needed before its true value can be determined. (Dr. Joseph J. Bunim and associates, Clinical Investigations)

LONG-TERM EVALUATION OF ARTHRITIS DRUGS CONTINUES

The long-term evaluation of the steroid drugs in the treatment of rheumatoid arthritis has been continued by Institute clinicians to further clarify their relative advantages and disadvantages.

The major synthetic corticosteroids now available to the physician are prednisone and prednisolone, triamcinolone, Medrol and dexamethasone. All of them are quite closely related chemically and represent the continuing attempts of the pharmaceutical chemist to develop drugs with greater therapeutic potency and reduced side effects. Prednisone and prenisolone are almost identical in therapeutic value and were the first of the synthetic steroids to be developed. They are approximately four to five times more powerful than cortisone and hydrocortisone and have almost completely replaced these older drugs in the treatment of rheumatoid arthritis. Perhaps their biggest disadvantage is their tendency to produce peptic ulcers, a side effect which, unfortunately, is not uncommon.

Triamcinolone and Medrol seem to offer few advantages over prednisone, although they may be useful in patients who experience particularly severe side effects from prednisone. Triamcinolone has been found especially useful in cases of rheumatoid arthritis complicated by psoriasis. Since all of the steroids have their own peculiar side effects, the availability of a number of them enables the physician to select the one best suited to the individual patient.

Dexamethasone is by far the most potent drug in the group and in the comparatively short time it has been under study has appeared to relatively free of major side effects. (Dr. Joseph J. Bunim, Arthritis and Rheumatism Branch)

STEROID DRUG THERAPY MASKS SYMPTOMS OF PEPTIC ULCERS

One of the recognized undesirable side effects sometimes caused by steroid therapy for rheumatoid arthritis is the production of peptic ulcers. A recent study at the

Institute has shown that many of the usual symptoms caused by these ulcers may be completely lacking in patients who develop them while receiving prednisone therapy for rheumatoid arthritis. Such valuable indirect signs of ulceration as localized tenderness and swelling surrounding the ulcer were sometimes completely absent, and even fluoroscopic examinations appeared normal in some patients with active ulceration.

In a group of 49 patients at the Clinical Center who were receiving prednisone daily 12 developed peptic ulcers during the course of the steroid therapy. Most of them had received other steroids for varying periods of time before starting on prednisone. The earliest ulcer appeared after three weeks of treatment, while one ulcer did not appear until six years of treatment, including hydrocortisone and prednisone. Thus it appears that peptic ulcers may develop after long periods of apparent "safety" with steroid therapy.

Because of these "silent" ulcers, every skill and technique should be used in an attempt to demonstrate the presence of an ulcer crater by all types of X-ray studies in any steroid-treated patients who experience even vague stomach pains, the investigators believe. (Dr. Roger Black, Arthritis and Rheumatism Branch and Dr. T. F. Hilbish, Diagnostic X-ray Department, Clinical Center)

NEW DRUG DISCOVERED FOR THE TREATMENT OF GOUT

An accidental discovery by Institute grantees has provided a potent drug for the treatment of gout. The compound is now undergoing clinical trial and appears to be a better uricosuric agent--one promoting the urinary excretion of uric acid from the blood--than any other now available. The drug itself, zoxazolamine (Flexin) is not new; it has been widely used as a muscle relaxant for several years. Its application in gout therapy, however, is the result of a chance observation during studies of the drug's metabolism in the body.

It was discovered that relatively large amounts of a crystalline compound appeared in the urine of patients receiving the drug, and although the crystals were first thought to be some metabolic product of zoxazolamine they proved to be uric acid. It was this observation that gave the first clue to the muscle relaxant's potent uricosuric properties.

Preliminary tests of the drug were then made in gout patients since a characteristic of their disease is the accumulation of excessive amounts of uric acid in the blood. The drug was found to have a considerably greater effect than any others available and appeared to be three times more potent than Benemid, the most effective uricosuric drug now in use for the treatment of gout. An added advantage over Benemid is that the action of the new drug is not inhibited

by aspirin. Further studies are continuing with zoxazolamine in gout patients at the Clinical Center since more experience with it will be needed before its full value can be determined. (Dr. J. E. Seegmiller, Arthritis and Rheumatism Branch)

BIOCHEMICAL STUDY EXPLAINS RAPID STEROID METABOLISM

Basic biochemical studies of steroid metabolism have now provided an explanation for the fact that hyperthyroid persons--those with over-active thyroid glands--metabolize or break down steroid hormones in their body at a faster rate than do persons in normal health. Such information is of considerable importance to rheumatic disease research since more knowledge is needed about the action of steroids like cortisone and hydrocortisone and how they are metabolized in the body

The fast metabolism of steroids was first noticed during clinical studies at the Institute and the phenomenon was then investigated in the laboratory to determine its underlying causes. It appeared that the over-active thyroid gland was speeding steroid breakdown in two ways; first by causing an increase in available TPNH, a biological reducing agent in the body, and second by increasing the amount of a steroid-reducing enzyme which was found in liver cells and named delta 4-3-ketosteroid hydrogenase (5 alpha). This enzyme, along with its co-factor TPNH, accomplishes one of the first steps in the steroid breakdown process, that of reducing a double chemical bond in the steroid molecule. This double bond in the molecule is necessary for the antirheumatic activity of adrenal cortical hormones and when it is reduced the molecule loses its characteristic properties.

These basic findings contribute to our understanding of the ways in which hormones and enzymes interact in the body to regulate important metabolic processes. The studies have also demonstrated the intimate interrelationships of various endocrine hormones, particularly those of the thyroid and adrenal glands. (Drs. Joseph McGuire, Jr., and Gordon Tomkins, Arthritis and Rheumatism Branch)

STUDY OF COLLAGEN FORMATION CLARIFIES ROLE OF VITAMIN C

The role which vitamin C (ascorbic acid) plays in the formation of collagen has been further clarified by a study of connective tissue growth in guinea pigs, done by a grantee at the Massachusetts Institute of Technology.

Collagen is one of the basic constituents of connective tissue and detailed studies of it are important in rheumatic disease research. Although little is known about the biochemical processes involved in its formation, past research has indicated that ascorbic acid plays a prominent role in the early stages of collagen synthesis. What has

not been known, however, is whether the vitamin's action takes place locally, such as at the site of a wound, or whether it is due to some general systemic effect.

Studies with plastic sponge implants in guinea pigs have shown that the vitamin does in fact act locally to speed collagen formation. The plastic sponges were implanted under the skin to act as a scaffolding on which new collagen could form, and some of the implants were given injections of ascorbic acid while others were not. It was found that collagen formed much more rapidly around the ones injected with vitamin C. (Dr. Bernard S. Gould, Massachusetts Institute of Technology, Cambridge, Massachusetts)

IRISH MOSS USED TO PRODUCE COLLAGEN

Studies of connective tissue metabolism have been greatly aided by a technique for inducing rapid collagen formation in guinea pigs with an extract of the seaweed known as Irish Moss.

The technique was originally developed by an Institute grantee who found that injections of carrageenin, a sulphated polygalactose present in the seaweed, would cause the formation of a collagen-filled growth of connective tissue under the skin at the site of the injection. The technique provided an experimental tool that has now become invaluable for the detailed study of collagen and its formation.

Other investigators found that the carrageenin injections result in the formation of connective tissue which appears on the 5th day in the form of fine fibers and matures by the 14th day into adult collagen. This is later broken down by the body and completely absorbed after 6 weeks. During the absorption process, however, the collagen fibers that had originally been present in the overlying skin are also lost, and are replaced by fat tissue. This reaction raises the possibility that carrageenin might be used therapeutically to remove scar tissue from the body, but much more study will be needed before such an application of the material is attempted. (Dr. W. van B. Robertson, University of Vermont, Burlington)

DIABETES

The future for the diabetic patient looks brighter now than it has for some time in the past. This is not to say that the long-sought cure will be discovered this week or next, nor does it mean that some slight improvement in the new oral antidiabetic drugs will finally free every diabetic from his need for insulin. What it does mean, however, is that research in diabetes is experiencing a new wave of interest. This interest has been spurred by the introduction of tolbutamide (Orinase) and the other new oral drugs, and has resulted in an extensive amount of new research, both into the mechanism of action of the drugs and, most importantly, into the basic causes of the disease itself. The increased interest has also been accelerated and maintained by the availability of added research grant support from the Institute in recent years.

Progress is being made. Fundamental studies in endocrinology, biochemistry and metabolism are all contributing new facts, and new theories, about the disease. Both of these are needed if the ultimate goals--prevention and cure--are to be realized.

OTHER NEW ORAL ANTIDIABETIC DRUGS DEVELOPED

Tolbutamide (Orinase), the first of the new oral antidiabetic drugs, came on the market in June, 1957, and has now been followed by several others. In the comparatively short time since their introduction an estimated half million diabetics--approximately one-third the known cases of diabetes in the United States--have switched from insulin injections to the new tablets.

Within the past year another new oral antidiabetic drug received extensive clinical trial and became available to the public in October, 1958. The new drug is chlorpropamide (Diabinese) and it is already in use by thousands of diabetics. Chlorpropamide has a slightly increased potency and can be given in smaller doses. Since it is excreted less rapidly than tolbutamide, the new drug may provide a smoother control of the level of blood sugar.

Both tolbutamide and chlorpropamide are sulfonylurea compounds. These compounds have been able to replace insulin injections in certain cases of diabetes because, like insulin, they have the ability to lower the high level of sugar in the blood and consequent urinary loss of sugar which is characteristic of the disease.

Exactly how the sulfonylureas accomplish this is not fully understood. Indeed, the more that is known about the drugs, the more complex the entire problem becomes. The weight of evidence to date indicates that they act by stimulating the beta cells of the pancreas to produce more insulin. Interesting evidence is accumulating, however, which

stresses the difference between their action and that of insulin with significant effects upon the liver and upon peripheral tissues such as muscle. Insulin appears to facilitate the passage of sugar across cell membranes into muscle tissue, but this action has not been demonstrated with the sulfonylureas.

In addition to tolbutamide and chlorpropamide which are now available to the public there are two other oral antidiabetic drugs which are still undergoing clinical trial before being made available to the public. These are phenformin (DBI) and metahexamide, and they will most likely be followed by still more in the future. Continuing pharmaceutical research is aimed at developing newer compounds which will be even less toxic than the older ones, have a greater degree of potency and be effective for longer periods of time.

Unfortunately, the sulfonylureas are not the "oral insulin" that has long been sought for the treatment of diabetes. They are effective only in those diabetics who contracted the disease relatively late in life and are of little use in cases of severe or "brittle" diabetes which usually begins in childhood or the early adult years. In this type of diabetes the beta cells of the pancreas can not supply the amount of insulin needed by the body and appear to be unable to respond to any stimulation.

Scientists at the Institute and grantees at non-federal research centers are continuing their efforts to determine the pharmacology, long-term effects and mode of action of these new drugs. In addition to their proven value in the treatment of diabetes, the drugs have provided a new and extremely valuable research tool by which many of the complex problems presented by the disease may be finally solved.

SMALL-DOSE CORTISONE TEST PREDICTS FUTURE DIABETICS

A new test for predicting whether relatives of diabetics will later develop the disease themselves has been given a preliminary screening by NIAMD grantees at the University of Michigan Medical School and found to be capable of detecting "prediabetes" with considerable accuracy.

The test is known as the small-dose cortisone test and consists of giving a small oral dose of cortisone followed by the standard glucose tolerance test for diabetes. If the cortisone causes a diabetic response to the glucose tolerance test it indicates that the person will probably develop the disease at some future time. The amount of cortisone given is so small that almost all normal persons fail to show any significant diabetic response after receiving it. A large enough dose of cortisone, on the other hand, always causes a shift toward the diabetic state, even in normal persons.

In families in which diabetes occurs, some members are normal and some are frankly diabetic. Between these two extremes are those showing poor glucose tolerance (a characteristic of the diabetic) while not being frankly diabetic, and those who react normally to the glucose tolerance test unless they are given cortisone. Since earlier studies had indicated that cortisone might tend to "unmask" a latent diabetes in those having a family history of the disease, the small-dose cortisone test was devised and administered to relatives of diabetics.

Follow-up studies were done to see how many subsequently developed the disease and revealed that approximately 12% of the relatives who had appeared normal with the ordinary glucose tolerance test but who had shown impaired glucose tolerance after the small-dose cortisone test later developed diabetes.

Although this preliminary screening of the test in over 500 individuals indicates that it provides an extremely reliable index of "prediabetes", another five-year follow-up will be necessary before final conclusions about the test's value can be drawn. (Drs. Jerome W. Conn, S. S. Fajans and associates, University of Michigan Medical School)

STUDY SHOWS LIVER INACTIVATES SOME OF BODY'S INSULIN SUPPLY

Institute scientists have found indications that the liver may be degrading or inactivating a considerable amount of the body's insulin supply before it is able to reach the tissues that need it. A precise knowledge of how much insulin is being destroyed in this way is of considerable importance to research in diabetes since the disease occurs either when there is too low an output of insulin by the pancreas or when too much insulin is inhibited or destroyed somewhere in the body.

The liver's effect on the insulin supply is particularly important because the organ occupies such a strategic position in the body. All insulin produced by the pancreas must first pass through the liver before being circulated in the body via the blood stream. Thus, any insulin degradation going on in the liver could have a decided effect on the overall diabetic state.

The Institute scientists were able to measure the total amount of degradation occurring in rat livers by using a technique known as organ perfusion in which the liver was isolated from its normal source of blood and supplied with a steady flow of fresh rat blood from a special perfusion apparatus. In this apparatus the blood is oxygenated much as it would be in the lungs, then pumped through the liver, and returned for more oxygen.

Measurements made of radioactive insulin injected into this closed circulatory system showed that in one passage of blood around the circuit 22% of the insulin disappeared, indicating that this amount of the hormone was being held by the liver. Increasing amounts of degraded insulin then began appearing in the blood from the liver in subsequent cycles. The biological significance of this insulin degradation is now yet fully known, and other studies have been designed to provide more information about this process, and how it may affect sugar metabolism in both the normal and diabetic person. (Dr. Glenn Mortimore, Laboratory of Biochemistry and Metabolism)

INSULIN FOUND TO CROSS PLACENTA IN ANIMALS

What is probably the first conclusive demonstration of the passage of insulin across the placenta from mother to unborn child has been reported by an investigator at the Harvard Medical School. The study, which was done in pregnant rats, supplies fundamental information about the permeability of the placenta and the interrelationship of hormones circulating in the mother's blood and in the blood of the developing fetus.

Past attempts to show whether insulin passed from the maternal to fetal circulation have been inconclusive. The present work was done to re-examine the problem and involved injections of insulin into the uterine artery of pregnant female rats. When blood samples were later taken from the fetuses, it was found that the injections had caused a marked decrease in the amount of fetal blood sugar, indicating that the insulin apparently crossed the placental barrier and exerted a hypoglycemic action in the fetus. (Dr. Ernest Knobil, Harvard Medical School, Boston, Massachusetts)

GASTROENTEROLOGY

Within the past year the Institute has greatly increased its support of research in gastroenterology, the medical specialty that deals with diseases of the stomach and intestines. Research in gastroenterology has been relatively neglected in past years although such diseases as peptic ulcer, ulcerative colitis and regional ileitis afflict thousands of Americans. Peptic ulcer alone is estimated to afflict 10 percent of all American adults at some time during their life.

These disorders are extensive in their effects and in many cases cause prolonged and expensive hospitalization, loss of income and countless social and emotional problems. Although they strike persons of any age, the younger and middle-aged groups are most severely affected.

A greatly increased number of research grants have been awarded by the Institute for the study of various aspects of intestinal physiology and pathology, including investigations of the changes in gastric secretions resulting from peptic ulcer, studies aimed at developing improved treatments for gastric duodenal hemorrhage, and the development of improved methods of diagnosis. These studies have produced many important results as the following items indicate and have served to stimulate interest in a field which until now has suffered from the lack of available research funds and interested investigators.

RADIO PILL BROADCASTS INFORMATION FROM INSIDE THE INTESTINAL TRACT

Important new information about the physiological processes taking place inside the stomach and intestines will result from the use of a new "radio pill" which is small enough to be swallowed and which broadcasts information from inside the body. The new device was developed by grantees at the Cornell Medical Center-New York Hospital and is now being used to study bowel motility.

The radio pill consists of a pressure-sensitive capsule containing an FM transistor radio transmitter and a tiny battery with a life of 24 to 30 hours. The entire device is about the size of a large vitamin capsule and causes no discomfort when inside the body. It can transmit pressures over long periods of time from the stomach or any segment of the bowel without the need for connecting wires or the insertion of the long tubes required by earlier methods.

The chief advantage of the new device is that studies of motility can now be made in areas of the body that have been almost inaccessible to other methods, such as the far end of the small intestine and the

right side of the colon. (Drs. John T. Farrar and James S. Bernstein, Cornell Medical Center-New York Hospital, New York)

MOTION PICTURES MADE DURING X-RAY EXAMINATIONS

A new, safe method for making motion pictures during fluoroscopic X-ray examinations of the intestines has been developed at Northwestern University Medical School. In the past, motion pictures of fluoroscopic examinations have been impossible. Examinations had to be limited to brief, few-second intervals of visualization on the fluoroscopic screen, because of the radiation danger from too long an exposure to X-rays. There was no chance to make a permanent cinematographic record of what was seen.

By devising a special timing mechanism for the fluoroscopic tube which turns it on for only a thousandth of a second at a time, the grantees have been able to keep radiation exposure to a minimum and still permit motion pictures to be made for periods as long as twenty minutes. The timer produces 30 of the millisecond bursts every second, a speed which coincides with that of the movie film in the motion picture camera attached to the apparatus. Extremely sensitive film and improved fluoroscopic equipment were also developed to make the new technique possible.

The fluoroscopic movies will now provide much more definite information about intestinal motility and the types of contractions appearing in various parts of the intestinal tract. (Dr. Gaston Van Trappen and associates, Northwestern University Medical School, Chicago, Illinois)

NEW METHOD IS DEVELOPED TO MEASURE FAT ABSORPTION

Children with cystic fibrosis often are unable to properly absorb fats due to the lack of sufficient digestive enzymes from the pancreas. Thus, they can become severely undernourished although plentifully supplied with food.

A new method to measure the rate of fat absorption, as well as changes in that rate, has been developed and is expected to be a useful tool in the study of cystic fibrosis, as well as other disorders affecting fat absorption.

The new technique involves giving patients a test meal containing radioactively-labeled iodine in corn oil. Measurements of the radioactive residue in stools and urine samples give researchers a measurement of the rate of fat absorption, and provide this information much more quickly than older and more involved balance studies.

Infants and children with cystic fibrosis and other conditions causing abnormal fat absorption can be studied with the new method to determine their rate of fat absorption both before and after the addition of pancreatic extracts to stimulate digestive activity. (Dr. Samuel Spector and associates, Western Reserve University, Cleveland, Ohio)

STOMACH BALLOON USED TO CONTROL INTERNAL BLEEDING

A useful new tool to control extensive bleeding in the stomach which would otherwise require surgery has been developed by clinical investigators in Minneapolis.

The apparatus consists of a rubber balloon positioned in the patient's stomach and connected by tubes to a refrigeration unit and pump. The unit circulates cold water through the tubes and balloon to chill the stomach wall. In 12 out of 13 cases of massive upper gastrointestinal hemorrhage, the device not only controlled bleeding, but slowed down the secretion and activity of gastric juices.

This method gives surgeons a useful means of controlling hemorrhages in the stomach both before and after ulcer operations. (Dr. O. H. Wangensteen and associates, University of Minnesota Medical School, Minneapolis)

RESEARCH ON GASTRIC JUICE UNCOVERS TWO NEW SUBSTANCES

Although the gastric secretions of the stomach are of vital importance to food digestion and are thought to play some part in the production of peptic ulcers, the chemical makeup of gastric juice is still poorly understood. This is due, at least in part, to the fact that gastric juice is consistently contaminated by other internal secretions and thus presents a difficult problem to biochemists attempting to obtain it in its pure form.

An analytical study of gastric juice by an investigator at the Oklahoma Medical Research Foundation has resulted in the isolation of a previously unknown enzyme which has now been named gastricsin. This enzyme belongs to a class known as proteolytic enzymes which have the ability to break down or "digest" protein material. The newly isolated enzyme accounts for fifty percent of the proteolytic activity of gastric juice. Pepsin, another proteolytic enzyme found in gastric juice, accomplishes the first stage of protein digestion by breaking the protein molecules into peptides. These peptides are then further digested by trypsin and other gastric enzymes.

The study has also indicated the presence of a substance in gastric juice which inhibits the secretion of hydrochloric acid in the stomach. Current work is directed toward isolating this active factor so that its composition and chemical properties can be determined. (Dr. Ranwel Caputto and associates, Oklahoma Medical Research Foundation, Oklahoma, City)

BASIC RESEARCH

The value of basic research has become increasingly apparent from recent advances in medical science. What might once have been considered interesting but non-practical pieces of laboratory research are, with increasing frequency, providing information that can be directly applied to the treatment of disease.

Knowledge of physical chemistry has aided the development of the steroid drugs for rheumatoid arthritis, where the replacement of one or two atoms on the steroid molecule can more than triple its potency. Basic studies of the metabolic break-down of a muscle relaxant drug in the body have led to the discovery of a potent new drug for gout. The finding that a few white cells circulating in the blood can synthesize DNA may have important implications in the study of how the body repairs radiation damage.

Basic research has always been of major importance to the National Institute of Arthritis and Metabolic Diseases. Here scientists are intensifying their research into the intricate relationship between hormones, enzymes and vitamins in the body to see how they affect metabolic processes which are vital to life and health.

Physical biology, a relatively new science has received increasing support from the Institute because of the growing realization that many complex biological problems will not be solved until we know what forces are operating in inter-molecular reactions. Thus the techniques and instrumentation of modern physics are being focused on areas that were recently the sole domain of the physiologist or the classical biochemist.

Today, medical research has matured to the point where future advances must depend more and more heavily upon basic research. It is from this substructure of basic research knowledge that tomorrow's conquests of disease will stem.

TWO NEW TESTS FOR FOLIC ACID DEFICIENCY DEVELOPED

Two new tests for folic acid deficiency in the body have been developed at the Institute which will be useful in determining the presence and extent of a dietary deficiency of this vitamin. The tests may also aid in determining the effectiveness of certain types of treatment against cancer.

Folic acid is one of the newest of the vitamins to be discovered and has already proved to be of great importance in clinical medicine. It is present in green leaves and is known as one of the anti-anemia vitamins, since it is able to promote the regeneration of red blood cells. Institute scientists have developed two sensitive tests for

the vitamin based on measuring, in urine, the amount of a substance known as formiminoglutamic acid. The tests are the outgrowth of earlier research by other NIAMD investigators which showed that if rats are made deficient in folic acid the amount of formiminoglutamic acid in their urine increased. Now by measuring the exact amount of it in the urine, the degree of folic acid deficiency can be determined.

The tests may also be useful in cancer studies since one form of treatment now being used is the administration of anti-folic acid compounds to help kill cancer cells. By measuring the folic acid balance in the body, it may be possible to determine how well the anti-folic acid compounds are doing their job. However, much more work with the tests will be needed before their full value will be known. (Dr. Herbert Tabor and Lillian Wyngarden, Laboratory of Pharmacology and Toxicology and Dr. Milton Silverman and associates, Laboratory of Nutrition and Endocrinology)

AMMONIA INTOXICATION TREATED WITH ARGININE, AN AMINO ACID

Arginine, one of the amino acids, has been successfully used in the treatment of ammonia intoxication by surgical investigators at the University of California School of Medicine.

Ammonia intoxication in the body occurs when the level of ammonia circulating in the blood becomes too high. It causes mental confusion and other neurological signs which will progress to complete unconsciousness and death if not properly treated. The ammonia is produced in the gastrointestinal tract by the action of bacteria on ingested protein substances, and under normal conditions is made non-toxic by biochemical reactions in the liver. The action of the normal liver is so efficient that usually little or no ammonia escapes into the blood stream.

When the liver becomes diseased, however, its ability to detoxify the ammonia is impaired, resulting in the accumulation of dangerous amounts of ammonia in the blood stream.

The investigators have found that intravenous injections of arginine increase the liver's production of urea, a compound which contains ammonia, and thereby cause a lowering of the ammonia level in the blood. The arginine injections, along with other therapeutic agents such as antibiotics to reduce bacterial growth, were used in 64 patients with ammonia intoxication and they brought about prompt lowering of blood ammonia concentrations as well as improvement in the patient's mental condition. (Dr. J. S. Najarian and associates, University of California School of Medicine, Berkeley, California)

PAIN KILLER MORE POWERFUL THAN MORPHINE GETS CLINICAL TRIAL

A potent new analgesic (pain-killing) drug developed by NIAMD chemists is now undergoing preliminary clinical trials at several clinics and addiction studies at the Public Health Service's Addiction Research Center at Lexington, Kentucky.

The drug, known as NIH 7519, is a total synthetic, made from derivatives of coal tar and belonging to a new series of compounds known as the benzomorphans. As a pain-killer, it has proved to be 10 to 20 times as potent as morphine among Laboratory animals, and in human patients following operations it afforded satisfactory pain relief at only one-tenth the dosage that would be necessary with morphine. In addition, the new drug appears to cause less respiratory depression than would be expected with morphine.

Scientists have been attempting for many years to separate the pain-relieving and the addicting characteristic seemingly inherent in substances having potency as great as or greater than morphine. In the process they have produced numerous drugs with wide variations in pain-killing power. Generally, however, it has been found that the greater the analgesic potency, the greater also are addiction and other harmful effects. Quite naturally, these undesirable properties have greatly limited the usefulness of these drugs.

NIH 7519, however, with pain-killing power at least ten times that of morphine, gives preliminary promise that a partial separation of the analgesic and addicting properties may have been achieved. In man, early findings have indicated that the drug is addicting, but the degree of addiction liability has not been exactly determined. The clinical studies must continue for some time before final conclusions about the drug's true value and safety are definitely established.

The new synthetic is the result of many years of systematic exploration in the Institute's laboratories. The addiction studies in monkeys were conducted at the University of Michigan. Chronic toxicity and other studies, including clinical trials of the analgesic's pain-killing ability are being carried on by Smith, Kline and French Laboratories, Philadelphia, which is producing experimental quantities of the drug. (Drs. Everette L. May and Nathan Eddy, Laboratory of Chemistry)

MISSING BIOCHEMICAL STEP IN MAN PINPOINTED BY SUGAR METABOLISM STUDY

The missing biochemical step in man that makes him dependent upon a dietary source of vitamin C has been pinpointed by Institute scientists studying the metabolism of glucose, a form of sugar, in the body. Vitamin C is needed to maintain connective tissue, bone and tooth dentine, and when a severe deficiency of the vitamin occurs it causes scurvy, a disease characterized by bone lesions and multiple hemorrhages throughout the body.

Man differs from almost all other mammals in that he cannot synthesize the vitamin within his own body. Most mammals are able to produce vitamin C within their bodies from a substance known as L-gulonic acid, a product of glucose metabolism. This L-gulonic acid is biologically converted to both vitamin C (ascorbic acid) and xylulose, a five carbon sugar.

In man, monkeys and guinea pigs, however, no vitamin C is formed. The L-gulonic acid is present but it is converted only to xylulose; none of it goes to form ascorbic acid.

Institute researchers studying the biochemical conversion of L-gulonic acid, have now found that the acid is not converted directly into either ascorbic acid or xylulose but rather goes through at least one intermediate step in the process. They have isolated one intermediate product, a compound known as 3-keto-L-gulonic acid, which is produced from L-gulonic acid and then converted to xylulose.

It is believed that a second intermediate also exists, one which is produced from L-gulonic acid and then converted to ascorbic acid. Additional studies are being carried out in an attempt to isolate this and other possible intermediates. (Dr. Gilbert Ashwell, Laboratory of Biochemistry and Metabolism, and associates in the National Heart Institute)

PROGESTERONE DURING PREGNANCY MAY CAUSE MASCULINIZED INFANTS

Progesterone, a female sex hormone, has been used for the past 15 years to prevent the occurrence of miscarriages during pregnancy and has made it possible for many women to give birth to normal infants that would not otherwise have survived.

A study of such cases by a grantee at Johns Hopkins University has revealed, however, that the hormone sometimes causes partial masculinization of female infants before birth. In some of the infants the masculinization was advanced to the point where sex determination was uncertain without exploratory surgery.

In 21 cases studied all but three of the mothers had been given progestinic substances either orally or by injection, and in most instances

the medication had been started in the early weeks of pregnancy. The mothers whose infants were most highly masculinized were the ones who had received the largest daily doses of the steroid. In spite of the masculinization effect the investigators do not believe that the steroid should be abandoned in the treatment of threatened or habitual abortion. In many cases, the abnormality is so slight that no correction is required, and even in the most severe cases surgical correction is relatively simple.

Surprisingly, the effect has not been noted before this, but a possible explanation is that the steroids produce this effect only occassionally and in mothers who differ in some way from others, possibly by metabolizing the steroid in an abnormal manner. (Dr. Lawson Wilkins and associates, Johns Hopkins University School of Medicine, Baltimore, Maryland)

BASIC BLOOD STUDIES HELP EXPLAIN RED CELL PRODUCTION

Institute scientists studying red blood cells have found evidence of a previously unsuspected "regulator" of red cell production in the body. These cells are of vital importance because they transport oxygen from the lungs to all other body tissues and their rate of production is carefully controlled.

In the past it has been thought that red cell production was regulated only by the amount of oxygen that was reaching the tissues. If too little oxygen was being received, then additional red cells were produced and released into the blood stream in order to carry more oxygen.

Recent studies of red cell production in certain types of blood disorders have now indicated that a second regulator may exist which permits finer control of cell production. The oxygen-regulated one would be held in reserve to handle the large demands for new cells such as might arise from the loss of considerable amounts of blood. The scientists suggest that the second or fine-control mechanism involves a substance produced in the red cells themselves which normally inhibits the production of more red cells. Thus when minor bleeding occurs and only a relatively small number of red cells are lost, the amount of inhibitor in the blood stream would be reduced. This, in turn, would result in more cells being produced until the original number was restored. (Dr. Frederick Stohlman and associates, Laboratory of Pathology and Histochemistry)

CIRCULATING BLOOD CELLS FOUND CAPABLE OF DIVISION

A study of blood cell multiplication, done in collaboration with scientists at Brookhaven National Laboratory, has shown that a small number of white

cells circulating in normal blood can synthesize DNA (deoxyribonucleic acid) and must therefore be presumed capable of dividing and forming new cells. This finding is in conflict with the long-held belief that circulating blood cells are "mature" and no longer capable of dividing, and may aid research on leukemia, a blood disease caused by the uncontrolled multiplication of white cells in the body.

The finding was made with the aid of DNA autoradiography, a new technique which can be used to indicate when cells are in the process of synthesizing DNA. This material is found in the nucleus of the cell and controls the cell's hereditary characteristics. Before a cell can divide, it must synthesize more DNA so that enough will be available for the new cell.

By using a radioactive "labeling" method, the scientists were able to demonstrate that two to five white cells per thousand were synthesizing DNA and therefore presumably preparing to divide.

From these and earlier radiation studies it now appears that there are white cells in the peripheral blood which can repopulate damaged bone marrow. This discovery is an important development in the study of high energy radiation damage to blood cells and how such damage can be minimized or prevented.

The DNA technique has also been used to study blood from patients with leukemia in an attempt to characterize their disease more exactly. The scientists have found that leukemic blood shows a higher percentage of DNA-synthesizing white cells, but in at least one type of the disease, lymphocytic leukemia, the number of such cells is limited even in the acute phase of the disease and differs little from the number found in the chronic form. (Dr. George Brecher, Laboratory of Pathology and Histochemistry, and associates at Brookhaven National Laboratory)

LIVER WOUNDS REPAIRED WITH PLASTIC SPONGE

The surgical repair of liver wounds has always been one of the most difficult problems to confront the surgeon. Mortality rates from liver injuries are high and in a great majority of cases the primary cause of death is hemorrhage, excessive bleeding from the damaged tissue. Because of the liver's rich blood supply internal bleeding is a constant threat and is difficult to stop once started.

Surgical investigators at the University of Washington School of Medicine have now reported the successful use of a plastic sponge, known as Ivalon, to control the bleeding that occurred after experimental liver surgery in animals. Slice wounds were repaired by covering the cut surface of the liver with a tailored-to-fit sheet of the plastic sponge. While it was being stitched into place, bleeding was controlled by temporarily closing off the incoming blood

supply to the liver.

The plastic sponge brought about a rapid and permanent stoppage of blood from the cut or damaged liver surfaces. Follow-up operations were done weeks and months later, and showed that the plastic had been fully accepted by the body, causing no excessive foreign-body tissue reactions.

The sponge appears to be effective because of its physical structure, which resembles that of a very fine-gauge net. Blood clots form quickly in the tiny spaces in the sponge and create a lattice-work upon which connective-tissue cells can lodge and form a durable covering for the cut surface. (Dr. Thomas W. Jones and associates, University of Washington School of Medicine, Seattle)

DIAGNOSIS OF LIVER DISEASE AIDED BY NEW METHOD

The characteristic yellow skin of patients with jaundice is due to excess bile pigments in their blood and tissues. For over a hundred years physicians have known that liver disorders have been associated with increased blood levels of total bile acids but have been unable to correlate individual increases of bile acids with specific diseases.

Now a clinical investigator has devised a method of measuring the concentration of the two main bile acids in blood serum and has found that the ratio of one acid to the other indicates both the type and severity of liver disease.

In addition to showing whether the patient has jaundice due to bile duct obstruction or as a result of liver cell damage, the new technique also should prove helpful in forecasting the course of liver and gall bladder disorders. (Dr. James B. Carey, Jr., University of Minnesota Medical School, Minneapolis)

SODIUM BICARBONATE USED TO TREAT SICKLE CELL ANEMIA

Sickle cell anemia is an inherited blood disorder which is found predominately in the Negro race and gets its name from the twisted, sickle-like shape assumed by many of the red blood cells circulating in the body. The sickling is caused by an abnormal type of hemoglobin in the cell, known as S hemoglobin, and prevents the cells from passing through many of the smaller capillaries of the blood system. As a result, the cells often pile up, obstructing blood flow, causing clot formation and leading to the painful periods of "crisis" that are characteristic of the disorder.

Institute grantees in Boston, Massachusetts, have found that large

amounts of a solution of sodium bicarbonate, given intravenously, may relieve the pain and fever of the crisis by reducing the amount of sickling in the blood. The sodium bicarbonate treatment was developed after earlier research had shown that the acidity or alkalinity of the blood is an important factor in the amount of sickling that takes place. When the blood is alkaline the cells show less susceptibility to sickling than they do when the blood is acid.

A preliminary study of the treatment was made in six patients with sickle cell anemia during crisis. The infusion of sodium bicarbonate brought prompt relief from the symptoms of the crisis in four of the cases. Its ineffectiveness in the other two may have been the result of starting the treatment too late or failing to bring about general alkalosis in the body. More extensive studies in a large number of patients will be needed before the value of the treatment can be fully determined. (Drs. Mortimer S. Greenberg and Edward H. Kass, Boston, Massachusetts)

CYSTIC FIBROSIS NOT INEVITABLY FATAL STUDY SHOWS

A long-term study of over one hundred patients with cystic fibrosis by investigators at the Children's Medical Center, Boston, has shown that this disease is not inevitably fatal in infancy and early childhood as has been widely thought.

Cystic fibrosis has been considered a disease of children since its victims seldom lived to adulthood in the past. Most died within several months, and many from pneumonia, one of the respiratory infections common in this disease. The disorder is thought to be an inborn error in the functioning of many of the glands in the body, and mucus, tear, salivary and sweat glands may all be involved. The major symptoms of the illness result from the thickening of mucus secretions in the lungs and pancreas. This thickening causes difficulty in fighting lung infections and renders the pancreas unable to supply vital digestive enzymes to the intestines.

As a result of their 14 year study, the investigators have developed a system for clinically evaluating cystic fibrosis patients based upon general activity, findings on physical examination, nutritional status and chest X-ray findings. The system provides physicians with a means for establishing the degree of severity of the disease, and will aid in determining how effective the various forms of treatment are.

Although cystic fibrosis is still a serious and often fatal disease, improved forms of treatment have extended the life of many young patients. Almost half of the patients studied were over 10 years

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of age and some were as old as 16 years. Treatment includes the use of the newer antibiotics, pancreatic preparations and nutritional therapy, including vitamin supplements and high protein diets. (Drs. Harry Shwachman and Lucas L. Kulczycki, Children's Medical Center and Harvard Medical School, Boston, Massachusetts)

BLOOD CLOTTING STUDIES REVEAL ACTION OF THROMBIN

New light on hemophilia and other puzzling blood disorders has been shed by the recent discovery of how thrombin, the body's essential blood clotting enzyme does its work.

Blood clotting is a complex process involving a series of biochemical steps, each one dependent on a previous one. Before clotting can take place, blood platelets have to rupture and release certain factors. These, in turn, act on prothrombin in the blood and convert it into its active form, the enzyme thrombin. Without thrombin, no clotting can take place.

Once active thrombin is formed, it brings about the last step in the clotting process, conversion of fibrinogen into fibrin, the gelatinous substance that actually forms the clot. How it does this has not been fully understood until now.

By using a radioactive nerve gas scientists in the Laboratory of Physical Biology were able to find the "active site" on the thrombin molecule that is responsible for its action and determine how the enzyme (thrombin) operates to change fibrinogen to fibrin. Their studies showed that thrombin acts on fibrinogen by "chopping off" the ends of the fibrinogen molecules. These molecule "ends" carry negative charges, so while they are attached, the molecules repel each other and will not form clots. But when thrombin is present, the ends split off, the charge is eliminated, and the fibrinogen molecules clump together as fibrin, forming the clot. Now, for the first time, the specific peptide bonds which are split by thrombin to remove these fragments have been determined.

The investigators also found that the thrombin molecule may be much smaller than previously estimated, having a molecular weight of approximately 14,000. (Drs. Koloman Laki, Jules Gladner and associates, Laboratory of Physical Biology)

RESEARCH YIELDS NEW FACTS ABOUT MUSCLE CONTRACTION

A five year study of the physical and chemical structure of muscle tissue by physical biologists at the Institute has revealed that myosin, the protein long thought to be the essential contracting substance in muscle fibers is not just a single protein, but rather a combination

of several distinct proteins.

The importance of this finding is that it brings closer the full understanding of one of nature's greatest mysteries, how the chemical energy in food is converted to the mechanical energy used by the muscle when it contracts and performs work. To understand this conversion it is necessary to know what kind of molecules are involved and what happens to their physical structure when they contract.

The research has indicated that a muscle fiber contracts because some of its protein molecules change their shape and cause the fiber to shorten. Then when the molecules resume their original shape the fibers stretch out again and the muscle relaxes. Further studies of the proteins in myosin are expected to reveal more details about this contraction process and how it operates in both health and disease. (Drs. Koloman Laki, David Kominz and associates, Laboratory of Physical Biology)

NEW ELECTRONIC TECHNIQUE AIDS STUDY OF MOLECULAR STRUCTURE

Physical biologists at the Institute are now using an advanced electronic technique known as nuclear magnetic resonance (NMR) to study the fine atomic structure of matter. Such knowledge about how individual atoms are arranged inside a molecule is of great importance to scientists attempting to synthesize insulin and other biological materials. The basic structure of a compound must be thoroughly understood before it can be duplicated in the laboratory.

By the use of the new NMR technique Institute scientists can determine whether certain atoms and combinations of atoms are present in a molecule and also what positions the atoms occupy in relation to one another. Essentially, the method consists of placing the test substance in a strong magnetic field and bombarding it with radio waves. Certain radio frequencies are absorbed by the substance and these absorbed frequencies indicate what atomic particles are present and how they are arranged.

The new research tool has already been used to provide detailed information about hydrogen atoms and how they serve to bind molecules together. All proteins and many other substances are held together by these hydrogen bonds, many of which may be forming and breaking thousands of times a second. Even water could not exist as a liquid without hydrogen bonds, since the individual water molecules would vaporize.

The equipment needed for NMR studies is typical of much of that now used by physical biologists; it is highly complex and expensive. The magnetic field generated must be completely stable and not vary more than one part in a hundred million. The same degree of accuracy

is needed in controlling the frequency of the radio waves generated. (Drs. Edwin Becker and Urner Liddel, Laboratory of Physical Biology)

AIRWAY RESISTANCE DURING BREATHING MEASURED BY NEW DEVICE

A new device for measuring breathing airway resistance, developed by Institute scientists, is supplying important basic information about the physiology of respiration. Known as a "whole body" plethysmograph, the machine may prove helpful in evaluating therapy for asthma and similar conditions involving increased airway resistance.

The whole body plethysmograph, the only instrument of its kind that provides continuous measurements during both normal and abnormal respiration, is a sealed capsule or chamber just large enough to accomodate a human subject in a sitting position. The capsule is insulated on the outside and kept at body temperature on the inside by a special mouthpiece, his breath velocity and the pressure changes inside the chamber are automatically recorded.

The new plethysmograph has demonstrated the striking finding that oxygen uptake from the lungs into the blood stream is a continuous process, one that goes on even while the lungs remain motionless on breath-holding. The device has also shown that each heart beat creates a negative pressure which brings air into the lungs, an event masked by normal respiration. These processes had not been visualized in humans before the improved plethysmograph became available. (Drs. Heinz Specht, Roscoe Bartlett, and associates, Laboratory of Physical Biology)

Items of interest on Research Studies Conducted and Supported by

the National Institute of Dental Research

MICROBIOLOGY STUDIES

SIGNIFICANT DATA DERIVED FROM GERM-FREE STUDIES

The magnitude and complexity of the oral microbial flora has long stood as a natural barrier to the systematic study of the suspected causal relationship of bacteria to diseases of hard and soft tissues of the mouth.

Recognizing the great potential of the germ-free animal as a research tool ideally suited for studies in oral microbiology, the National Institute of Dental Research initiated a limited program in 1958 applying this technic to the study of the etiology and mechanism of caries, calculus formation, and periodontal disease.

It is now well established that germ-free rats do not develop caries even when maintained on diets which consistently produce severe caries in conventional animals. In a preliminary study by Drs. R. J. Fitzgerald and H. V. Jordan, Jr., of the Laboratory of Microbiology, NIDR, dental caries was successfully produced in germ-free animals by inoculating their oral cavities with a single pure strain of streptococci originally isolated from carious rats. The characteristics and severity of the resulting carious lesions were comparable to those seen in conventional animals. Further studies demonstrated that initial carious lesions can also be produced in rats mono-infected with diphtheroid microorganisms originally isolated from carious lesions. This finding suggests that the diphtheroids, hitherto ignored in considerations of caries etiology, warrant further study.

A further finding of potential significance was the observation of calculus-like deposits on the molars of some germ-free animals. Current concepts hold that the microorganisms

inhabiting the oral cavity are primarily responsible for calculus deposition and that this tartar, in turn, provides the initial irritant in the sequence of tissue changes leading to periodontitis (pyorrhea). Should these deposits prove to be closely related to the tartar seen in humans a significant breakthrough will have been made in our understanding of periodontal disease.

STUDIES OF THE IDENTIFYING CHARACTERISTICS AND GROWTH REQUIREMENTS OF ORAL BACTERIA

A study by Dr. Robert R. Omata of the Laboratory of Microbiology, NIDR, has elucidated certain of the nutritional requirements of oral microorganisms, among the more important being that certain vitamins and purines are required for growth. Evidence to date gives promise of aiding in the differentiation of the various types of fusobacteria and of clarifying their role in the oral microbial environment.

With reference to the incidence of pathogenic or potentially pathogenic fungi from various sites within the oral cavity, recent studies by Dr. Arden Howell, Jr. and M. Rogosa, Laboratory of Microbiology, NIDR, have led to the isolation of an unusual and hitherto little known organism known as the Leptotrichia buccalis. Essentially anaerobic, branching, and filamentous in form, these organisms are being evaluated by investigators at both the NIDR and at Duke University School of Medicine to determine their specific identity, natural taxonomic position, pathogenicity, and significance, if any, in relation to caries and calculus formation.

IMPROVED BLOOD CULTURING TECHNICS DEVELOPED BY NIDR

Clinical studies in cooperation with the National Heart Institute have developed improved methods for detecting the presence of bacteria in the blood stream following exodontia and periodontal treatment. Results, using modified blood culturing technics, indicate that present estimates of the frequency and extent of bacterial invasion following oral procedures are far too low. A total of 67 cases have been studied to date with an overall incidence of 57, or 85 per cent, positive blood cultures after dental surgery and periodontal treatment. These results represent a two-fold increase over those found by routine current methods, and makes available a technic which will provide clinical laboratories with optimal procedures for a more critical biologic assessment of blood. Particular benefits may thus accrue to patients with rheumatic fever and other heart disorders.

BIOCHEMICAL STUDIES

THE CARIES INHIBITING EFFECT OF PHOSPHATE MINERALS

From studies by Dr. F. J. McClure, Laboratory of Biochemistry, NIDR, it has been reported that certain dibasic phosphates show considerable promise as effective anti-caries agents.

Current and past investigations at NIDR, using sodium acid phosphate as a dietary supplement to wheat cereal, bread and drinking water, have consistently demonstrated an inhibiting effect on both the severity and incidence of carious teeth in test animals. These data suggest that the caries protective effect may be a localized one within the oral cavity rather than one mediated through systemic channels. Preliminary clinical studies are under way to provide confirmatory evidence of this protective effect of dietary phosphates and to evaluate its possible application as a dental public health measure.

THE EFFECT OF AMINO ACID (LYSINE) DEFICIENCY ON CALCIFICATION

In a series of experiments using laboratory rats, Dr. R. C. Likins of the NIDR Laboratory of Biochemistry, Dr. L. A. Bavetta of the University of Southern California, and Dr. Aaron S. Posner, Research Associate of the American Dental Association at the National Bureau of Standards, studied the effect of amino acid deficiency on mineral metabolism. Lysine was the essential amino acid used since it is known to be required for normal skeletal growth. Studied by means of radioactive calcium, it was found that an increase in dietary lysine is accompanied by an increased deposition of the radioactive calcium in bone. Further observations showed that the radioactive calcium content in the presence of a lysine deficiency could not be related to chemical or physical changes in the bone per se, but rather appeared to be the result of an inability of the test animals to retain their skeletal deposits of calcium. These findings indicate that a lysine deficiency, with accompanying loss of calcium, results from failure of proper bone growth.

ENZYMATIC CONVERSION OF ETHANOLAMINE

Scientists working in the Laboratory of Biochemistry, NIDR, have isolated a bacterium from nature which metabolizes a simple organic compound (ethanolamine) for its sole source of structural material and energy. Cell free preparations of this organism have been shown to convert ethanolamine to glycolaldehyde, which appears to be the first step in the metabolic utilization of the compound. This

investigation is one in a series designed to study the nature of simple organic compounds and better our understanding of their role in the formation of the more complex amino acids and protein structures associated with the oral cavity.

SYSTEMIC FACTORS LINKED WITH CARIES PROCESS

In studies to correlate the nutritional state and physical well-being of animals with the incidence of dental caries, NIDR grantees, Drs. R. R. Steinman and M. C. Hardinge, College of Medical Evangelists, California, designed a dual experiment to determine, in the posteruptive period, whether cariogenic or noncariogenic diets influence nutritional absorption in the dentin of teeth, and whether the caries process is affected by varying quantitatively specific nutrients in the diet.

Using a dye, trypan blue, injected intraperitoneally to determine nutrient absorption within the dentin, it was found that animals fed a noncariogenic diet exhibited a greater concentration of dye in dentin tissue than did those animals maintained on a cariogenic diet.

To evaluate the effect of caries production by quantitatively varying specific nutrients, two equal groups (by litter) of animals were established. One group was fed representative foods of an average American diet (minus dessert) while the other group received the same diet supplemented with an equal mixture of glucose and fructose administered intraperitoneally three times daily. After 13 weeks all animals were sacrificed and examined for caries.

Data obtained from these studies indicate that nutrient absorption within the dentin is perceptibly different in animals fed cariogenic and those fed noncariogenic diets. Further, it was demonstrated that parenterally administered sugars significantly increased the incidence of caries in the experimental animals. In light of these results it would appear that in addition to the adverse influence of the bacterial flora and fermentable carbohydrates in the mouth, a variety of systemic factors, involving a metabolic disturbance of the host, also contributes materially to the caries process.

BASIC CELL RESEARCH MAKES SIGNIFICANT PROGRESS

The nucleic acids found in basic cell structure are thought to carry the pattern of living matter from one generation to the next.

In an attempt to understand more fully the role played by these compounds in oral health and disease, scientists in the Laboratory of Biochemistry, NIDR, have reported success in the conversion of one nucleic acid, ribonucleoside (RNA), to another, deoxyribonucleoside (DNA) using the bacteria Salmonella typhimunium. Both of these compounds, which are almost identical in molecular structure, are implicated with the hereditary factors found in human cells and are also known to be involved in the development of proteins in the human body.

BASIC BIOCHEMICAL FLUORIDE STUDIES SHED LIGHT ON HOW TEETH AND BONE ARE AFFECTED

Following the establishment of the value of fluorides as a major factor in preventing tooth decay, there has been speculation as to how this beneficial action is accomplished. The object of a series of investigations in the Laboratory of Biochemistry, NIDR, has been to understand better the manner in which fluoride acts on the various inorganic compounds of mineralized tissue.

Using fresh and ashed bone it was found that a radioactive phosphorus exchange with the ashed bone is complete in approximately 48 hours, whereas a period of a month is required for a similar degree of exchange using fresh bone. In contrast, the simultaneous use of fluoride and radioactive phosphorus showed that while concentrations of two and ten parts per million fluoride increased the rate of exchange of the radioactive phosphorus in enamel, dentin and bone, a concentration of 100 parts per million fluoride appeared to retard this action.

Data obtained from this study indicates that (1) the building of mineralized tissue or mineral exchange is not accomplished entirely by recrystallization, but rather that the organic matrix of bone, enamel and dentin is involved in this action, and (2) the presence of fluorides affects this rate of exchange.

INHIBITION OF LYSINE UTILIZATION

Lysine, an essential amino acid, and lysine peptides have long been recognized as nutritional factors which contribute not only to the growth of animals but also to the growth of lactic acid bacteria.

Basic research studies at the NIDR have provided additional data on the manner in which these compounds are utilized in the process of protein synthesis. In a study by Drs. T. Shiota and J. E. Folk, Research Associates of the American Dental Association working at NIDR, and Dr. F. Tietze of the National Institute

of Arthritis and Metabolic Diseases, specific lactic acid bacteria were used in conjunction with a lysine inhibitor, S-(β-aminoethyl)-cysteine (AEC), to observe the manner of lysine utilization. The results showed this agent to be an effective inhibitor of growth for lactobacilli and indicate that certain peptides containing lysine were more effective than free lysine in providing for the growth of these bacteria. These results are of importance in elucidating the manner in which peptides are utilized for protein synthesis in the living cell.

NON PROTEIN ORGANIC SUBSTANCE IDENTIFIED IN DENTAL ENAMEL

Human enamel, the hardest substance found in the body, consists mainly of inorganic materials (96 percent) and only a very small amount of organic substance (1.7 percent); the remaining 2.3 percent being water. This relatively small amount of organic material, not demonstrated previously and heretofore considered to be almost exclusively protein of ectodermal origin, has been the object of considerable study by Dr. Gordon Nikiforuk, NIDR grantee at the University of Toronto, Canada.

Using cation exchange resins together with chelating agents, specimens were prepared and subjected to paper chromatographic analysis. The resultant yield produced evidence of the presence of the carbohydrates galactose, glucose, mannose, and fucose. These findings suggest that a small but important carbohydrate component of enamel structure may be implicated in the onset of carious lesions.

ENZYMATIC BREAKDOWN OF DENTAL MUCOPOLYSACCHARIDE MAY BE IMPLICATED IN FORMATION OF CARIES

Dental students working under the part-time NIH research fellowship program at the Emory University Department of Biochemistry have suggested the enzymatic breakdown of mucopolysaccharides in teeth as an explanation of the etiology of dental caries. The known strong resistance of enamel and dentinal protein to proteolytic digestion suggests that some other fraction in the protein molecules provides a point of attack.

Previous studies confirmed the presence of mucopolysaccharide identified in human enamel and dentin as chondroitin sulfate, a recognized constituent of cartilaginous tissue and bone matrix. To ascertain what action a crude enzyme preparation from bacteria associated with human dental caries might have on chondroitin sulfate, H. H. Hughston and L. Earle selected as test organisms a pure culture of oral streptococcus obtained from carious lesions

of dental patients. Using commercial chondroitin sulfate and crude chondroitin sulfate extracted from the teeth of cattle, bacterial enzyme material was added and found to cause hydrolysis of both chondroitin sulfate specimens. Further study may reveal this destruction to be a primary reaction in the formation of carious lesions.

THE USE OF VITAL STAINS AS SELECTIVE INDICATORS FOR BEGINNING DENTAL CARIES

Studies of dental caries in experimental animals have shown that many factors affect caries susceptibility. These include conditions which influence tooth formation and those which control the oral environment. Although vital stains have been used for many years to demonstrate phases of tooth and bone formation, recent studies at NIDR have shown their value as chemical indicators in the caries process and for early detection of dental caries.

In a series of experiments by Dr. R. M. Stephan, Clinical Investigations Branch, NIDR, a variety of vital stains were tested by injection and by addition to the drinking water and diets of rats. Several of these were found to selectively stain the earliest stages of caries, as well as be incorporated into teeth during their formation. Thus, considerable value may be related to their use as chemical indicators of caries activity.

HISTOLOGY AND PATHOLOGY

TRANSMISSION OF EXPERIMENTAL DENTAL CARIES

An extremely confusing aspect of the experimental caries picture in hamsters and rats has been the variability in caries activity between animals subjected to almost identical experimental conditions. This variability has often been wide even between animals in the same laboratory and so extreme in animals in different laboratories that results have seldom been reproducible.

Evidence is now available which indicates that caries in hamsters and rats is closely related to a transmissible bacterial flora and that the disease might therefore be considered contagious under present experimental conditions.

In one of a series of completed studies by Dr. Paul H. Keyes of the Laboratory of Histology and Pathology, NIDR, a critical test was performed to demonstrate the possible transmission of factors that may be involved in the variability of experimental caries. Thirty-eight caries resistant hamsters were divided into two

equal groups: one was placed in cages with hamsters of known caries susceptibility, while the other was kept isolated. Animals in both groups were maintained under identical conditions with respect to diet, water, and number of animals per cage (4). At the end of a 56-day experimental period it was found that in the exposed group, 94.3 percent of the teeth were affected whereas only 42 percent of the teeth in the isolated group showed caries. Furthermore, the lesions were severe in the former group but relatively minor in the latter.

These and other significant observations open for reconsideration the influence of the bacterial flora of the alimentary canal on the caries susceptibility of animals.

RADIOACTIVE TRACER STUDIES PROVIDE INFORMATION ON THE COMPARATIVE METABOLISM OF CALCIUM AND STRONTIUM

There is at present an acknowledged interest in the metabolism of radiostrontium. New data have been obtained on one aspect of this problem in a collaborative study by Drs. R. C. Likins of NIDR and A. S. Posner (ADA Research Associate) of the National Bureau of Standards. Using radioisotopes of calcium and strontium, the deposition, retention, and excretion of these ions has been traced in experimental animals.

In rats injected intraperitoneally with a solution containing labelled calcium and strontium, the initial skeletal uptake of these ions was found to be in the same ratio as that of the solution administered. Thus, in the formation of bone there was no apparent preference for either calcium or strontium. On the other hand, measurements of the relative retention of these ions during the first twenty-four hours after injection revealed that strontium was lost preferentially from the skeleton. Analyses of the urine collected for twenty-four hours revealed that the excretion of radiostrontium by the kidneys was approximately four times that of radiocalcium.

To investigate the preferential loss of strontium from bone, synthetic hydroxyapatite (the calcium phosphate prototype of bone mineral) labelled with radioactive calcium and strontium was prepared. Like bone, these synthetic materials released relatively more strontium than calcium when placed in unlabelled calcium solutions. This finding suggests that calcium is more stable in the hydroxyapatite structure than is strontium.

DISTRIBUTION OF RADIOACTIVE STRONTIUM IN HUMAN TEETH

NIDR grantees report new data bearing on the deposition of radioactive strontium in teeth and its possible relationship to fluorides.

The advent of nuclear weapons has stimulated interest in strontium metabolism because of the potential deleterious effect of skeletal fall out. Recent claims made by some antifluoridationists that water fluoridation is hazardous because fluoride may increase body deposition of strontium have been refuted by NIDR grantees at the Eastman Dental Dispensary, Rochester, New York. Their findings indicate that fluorides in drinking water do not affect the deposition of strontium.

Current and previous studies have confirmed that radioactive strontium is readily taken up by dental structures and that acquisition is primarily during calcification of teeth prior to eruption. In contrast to bone, once strontium is deposited in a tooth the element is likely to become more permanently seated.

Studying extracted human teeth collected from widely scattered geographical areas in this and foreign countries, investigators at the Rochester laboratories have demonstrated that the deposition of fluorides and strontium 90 in calcified tissue of the mouth is independent and unrelated. This is supported by data which indicates that strontium exchanges chemically with calcium in the apatite crystal of enamel while the fluoride ions undergo exchange with the hydroxyl groups of the crystalline lattice. Thus, the assertion that fluorides present in tissue fluids of the body may cause precipitation of strontium fluoride in calcified tissues and thereby adversely influence the deposition of radioactive strontium, appears to be unwarranted.

SUB-MICROSCOPIC STUDIES OF DENTINOGENESIS PUBLISHED BY PHS

NIDR investigators have published an atlas dealing with an electron microscopic study of the early stages of dentinogenesis.

Drs. Marie U. Nylen and David B. Scott, Laboratory of Histology and Pathology, NIDR, have published an atlas reporting on one of the first embryological studies that has been made at the electron microscopic level.

The report (PHS Publication No. 613) is a comprehensive treatise that extends beyond the area of histogenesis of dentin in illustrating the several structural changes undergone by a group of cells during their differentiation. In addition, it depicts the subsequent alterations that accompany the tissue-forming activities of these cells. Further, since the fibrillar elements that are elaborated as a result of cellular function are collagenous, the findings should merit the attention of those who are working in the currently active field of connective tissue biology.

Because of its breadth and systematic approach to an understanding of dental histogenesis, this publication should be of interest to teachers in the dental and basic sciences as well as researchers.

SIGNIFICANT RESULTS REPORTED IN TRANSPLANTING AND REPLANTING TEETH

NIDR grantee investigators at the University of Kansas City School of Dentistry have reported increasing success in the development of technics for surgical transplantation and replantation of full-term molar teeth in laboratory animals.

In studies demonstrating the transplantation of teeth, two groups of hamsters were used. In both groups the bilateral mandibular second molars of all animals were extracted and placed in sterile normal saline. Immediately thereafter the teeth of group 1 animals were transplanted to the sockets of group 2 animals while the teeth of group 2 animals were placed in sockets of group 1 animals. No special postoperative care was administered except that all animals were maintained on a specially devised "soft diet" for 3 days following oral surgery.

After 30 days all animals were sacrificed and mandibular sections were prepared. Resulting data revealed 100% "takes" in all transplanted teeth with approximately one-quarter classed as good "takes," where pulp, epithelium and periodontal membrane tissue exhibited capacity for complete regrowth.

In demonstrating similar technics for the replantation of teeth, bilateral second molars were completely dislodged and then replaced immediately in the original sockets. Observation of 30-day postoperative sections again revealed 100% "takes" with approximately one-half being classed as good. No evidence of tissue incompatability of antibody formation was detected in animals subjected to transplant or replant procedures.

NEW TISSUE FIBER DEMONSTRATED BY NIDR INVESTIGATOR

Dr. Harold M. Fullmer, Laboratory of Histology and Pathology, NIDR, has detected the presence of certain fibers believed to be a hitherto unidentified form of connective tissue.

During the course of investigations of age changes in connective tissue (including periodontal membrane, Achilles tendon and patellar ligaments of humans, monkeys, mice, rats and guinea pigs), it was found that with the usual staining procedures, the fibers appeared to be composed almost entirely of white collagen. However, with special histochemical staining technics (periacetic

acid and aldehyde fuchsin), Dr. Fullmer was able to observe segments of connective tissue differ from human components as regards morphological characteristics, chemical composition, and histophysiological function.

CLINICAL AND EPIDEMIOLOGICAL STUDIES

At this time the etiology of periodontal disease is not completely understood. While useful inferences on etiology may frequently be made on the basis of epidemiological studies, its particular long-range goal is control of disease. Historically, the field of epidemiology is the only discipline which has led to control of a disease process of unknown etiology.

EPIDEMIOLOGICAL STUDY RELATES PERIODONTAL DISEASE TO SOCIO-ECONOMIC STATUS

Drs. A. L. Russell, Epidemiology and Biometry Branch, NIDR, and Polly Ayers, Bureau of Dental Health, Jefferson County Department of Health, Birmingham, Alabama, have completed a study designed to examine the differences in periodontal scores obtained from white and Negro groups and to ascertain, if possible, whether resultant scores reflect a true racial difference or if they can be explained solely on the basis of socioeconomic status.

A total of 2,150 white and Negro persons, aged 15-74 years, were given periodontal examinations in Birmingham, Alabama. Based on analysis of this select heterogenous population, it was concluded that: (a) of the several factors studied, poor oral hygiene seemed most closely associated with the etiology of periodontal disease; (b) no significant differences in incidence or severity of periodontal disease could be demonstrated between Negro and white groups; (c) given relatively clean mouths and equivalent needs for dental care, persons of higher socioeconomic status showed less periodontal disease than did those persons with lesser education and income.

The test was programed to include as many persons of diverse background, education, and occupation as was practicable. Results obtained indicate that periodontal disease comparisons between populations should be based upon groups equivalent in socioeconomic status.

EPIDEMIOLOGICAL STUDY OF ORAL DISEASES IN ALASKA

In a coordinated study with the Interdepartmental Committee on Nutrition for National Defense,

and in cooperation with the PHS Arctic Health Research Center, NIDR investigators have recently completed a preliminary epidemiological survey of oral disease in Alaskan Eskimos.

At present the Eskimo lives under conditions ranging from the most primitive to highly civilized; a spectrum which affords a unique opportunity to study oral disease uninfluenced by professional treatment. Thus research efforts may be focused more directly on specific factors, such as nutritional deficiencies, that might be responsible for the high prevalence of dental caries and periodontal disease in civilized populations.

Preliminary examination of about 700 Eskimo men of the Alaska National Guard by Dr. A. L. Russell and C. L. White, Epidemiology and Biometry Branch, NIDR, revealed significant data regarding the oral health of this group. Comparing the prevalence of oral disease in individuals living under relatively civilized conditions with that of men from primitive villages, it was found that dental caries is virtually nonexistent in the latter group. Of further significance was the observation that many of these primitive groups were essentially free of periodontal disease. Although other male individuals from similarly primitive areas exhibited gingivitis, the disease apparently did not progress to destruction of deeper periodontal tissues even in the absence of personal hygiene or professional dental care. However, it was noted that when Eskimos have lived for some time under relatively civilized conditions, the prevalence of oral disease increases to a point quite comparable to that seen in average male population groups in the United States.

The expansion of epidemiological and biometric studies in 1958 to include selected population groups in India, Alaska and Ethiopia has contributed not only to a better understanding of oral disease patterns of prevalence and severity in the people, but also to the further development of testing of methods for assessment of periodontal disease.

GLAUCOMA DETECTION TEST RESULTS FROM NIDR GENETIC STUDIES

In a demonstration of how basic research studies are contributing to the broad field of health, the Human Genetics Section, NIDR, recently announced the development of a method for the prediction and early detection of glaucoma disease through study of inheritance patterns among kindred of affected individuals.

Based on observations by Mrs. Hazel R. Dyson, RN, it was found that if an individual has an inherited disease, examination of predetermined members of the kindred may reveal additional

examples of the disease and thus increase the effectiveness of screening procedures. Further, if the mode of inheritance is dominant, as with glaucoma, one can accurately trace the affected individuals through family history and home visits to identify the sibships that should be examined.

During the early stages of a current dental examination program of an isolate population group in Maryland, two patients were found to have chronic glaucoma. A family history of eye disease was obtained for each and the sibships in which glaucoma was most likely to occur was established. Examination of the selected kindred revealed five cases of frank glaucoma and one glaucoma suspect. Two of the patients were discovered before they were aware of any eye trouble. Additional studies on other families have shown that relatives of affected patients frequently have the disease.

Glaucoma in its initial stages is often more susceptible to treatment and damage from prolonged intraocular pressure is minimized if identified early. Detection tests now being offered by the Prevention of Blindness Society have shown Mrs. Dyson's method of screening to be an effective and practical public health measure for case finding.

X-RAY DOSES IN DENTAL ROENTGENOGRAPHY

Professor Albert G. Richards, NIDR grantee at the University of Michigan School of Dentistry, has shown in a recent study that complete roentgenographic examination of the human jaw results in a total dosage to gonadal tissue of approximately 2.14 mr. When this procedure is repeated 46 times, the total gonadal exposure is only 100 mr., or the same negligible amount that would be received from thirty years' exposure to the radiation resulting from atomic weapons at the present rate of testing. When it is considered that during the same thirty-year period, an individual is exposed to a total of 4,300 mr. from natural radiation, such as cosmic rays and radioactive minerals, it is evident that dental X-ray equipment for diagnostic purposes is well justified and creates a minimum of hazard when properly used.

Proper filtration, cone angle, target distance, X-ray film emulsion, and processing enabled the investigator to limit the amount of radiation reaching the gonads to 1/10,000 the amount delivered to the patient's face in producing the oral X ray; and the amount of total skin dose over the region of the maxillary bicuspid teeth, where dosage is heaviest, to 6.5 r for a complete examination.

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RELATIONSHIP OF ENAMEL HYPOPLASIA TO ABNORMAL EVENTS OF GESTATION AND BIRTH

NIDR grantees at the Henry Ford Hospital, Detroit, Michigan, have demonstrated a significant correlation between the time of occurrence of enamel hypoplasia and the time of occurrence of abnormal events of gestation and birth leading to cerebral disorders.

In studies to determine if a correlation exists between cerebral disorders and enamel defects, Drs. W. F. Via and J. A. Churchill obtained detailed information concerning gestation, birth and subsequent development from the parents and/or medical records of 312 children. On the basis of a neurologic examination, the patients were segregated into two groups, one with definite signs of cerebral dysfunction and the other with no indication of cerebral dysfunction. Only patients who had undergone dental examinations and whose teeth were such that it was possible to determine whether hypoplasia was present or absent were admitted to the study. Of the 312 children studied, 121 had enamel hypoplasia and also had experienced one or more abnormal events during gestation and birth. A strong correlation was found to exist between the time of occurrence of these dental defects and the abnormal factors related to gestation and birth.

HIGHLIGHTS OF PROGRESS

IN

MENTAL HEALTH RESEARCH

1958

Items of Interest on Research Studies Conducted and Supported by

the National Institute of Mental Health

This year saw the completion of a number of important studies on the biological and social bases of schizophrenia, personality development, the structure and function of the nervous system, and the biochemistry of behavior. The research literature also indicates a widespread concern with the effect of family structure and parental behavior on the behavior of both healthy and disturbed youngsters.

BIOLOGICAL STUDIES OF SCHIZOPHRENIA

Studies in the biology of schizophrenia have proceeded along four general lines this year. The search for a substance produced by the body, which clearly differentiates schizophrenics from non-schizophrenic individuals continues. Along with this, the study of chemically produced analogues of schizophrenic behavior continues to receive research attention. New studies of the electrophysiology of schizophrenia have been undertaken, and this year has seen the publication of results of certain long range studies evaluating such physiological treatments of schizophrenia as electroshock and brain surgery.

BIOCHEMICAL DEVIATIONS OF SCHIZOPHRENIA

Few research areas are as tortuous or as laden with experimental pitfalls as that which concerns itself with the Biology of Schizophrenia. The number of variables present in any complex biological study is for all practical purposes infinite. No two living things are exactly alike; no two biological events are ever exactly repeated. Not only is each individual unique, but he is constantly changing.

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Trying to find an orderly relationship between the appearance of biochemical substances and the schizophrenic condition is a very difficult undertaking. The history of this search is marked mostly by discoveries of apparent relationships which then turn out to be really unrelated chemical events. Each such finding is not negative, however, for each one identifies new sources of experimental variability and each narrows the area of search.

This year, NIMH scientists and grantees have conducted a number of studies which have made such contributions, and which illustrate the complexity of this area.

One hypothesis which appeared in the literature recently suggested that disturbances in the metabolism of adrenalin were characteristic of schizophrenics. Adrenochrome, a hallucinogenic drug which is a product of adrenalin was found by one experimenter to be present in larger than normal quantities in the blood of these patients. In repeating these experiments at NIMH, Dr. Stephen Szara and his associates found a source of possible variability in the standard technique of measuring adrenochrome. When more precise measuring techniques were developed, the relationship was shown to have been due to the measurement procedure rather than to schizophrenia, although that procedure had heretofore been the accepted procedure for measurement of adrenochrome.

Last year an investigator reported abnormal ascorbic acid levels in schizophrenics and also that ceruloplasmin levels of schizophrenics were unusually high. Dr. R. K. McDonald and his associates at NIMH repeated these experiments with certain additional experimental controls. He found that when the schizophrenic patients received regulated diets, ascorbic acid levels were related to the amount of ascorbic acid in the diet. By using a variety of control and measurement techniques, McDonald demonstrated that the reported abnormal ceruloplasmin levels were related to other biological variables rather than to schizophrenia.

Another report from a group of scientists at the University of Utah Medical School refutes earlier work by this group which indicated abnormal patterns of indole-acid excretion in schizophrenic patients. Similar abnormal patterns have been found in other patients, and administration of antibiotics resulted in disappearance of these substances from the urine.

Despite this succession of negative findings, the search for biochemical correlates of schizophrenia continues. Dr. Samuel Bogoch of Harvard Medical School's Department of Psychiatry has reported evidence that adult schizophrenics have considerably less neuraminic

acid in the cerebrospinal fluid than do nonschizophrenics. The levels of neuraminic acid of the schizophrenic patients Dr. Bogoch examined were "comparable only to values found in some children under seven years of age." Dr. Bogoch has advanced a tentative hypothesis that low concentrations of neuraminic acid might affect the functioning of the blood-brain barrier. An inadequately developed blood-brain-barrier system, he suggests, might result in a specific vulnerability to psychosis.

EXPERIMENTAL "PSYCHOSIS"

Studies of LSD (lysergic acid diethylamide), a chemical which produces transient schizophrenic-like behavior, have continued. NIMH grantees at Clark University report that both schizophrenics and normals, when under the influence of LSD, see body parts as significantly larger than they do when not under the drug's influence. At the same time, there is no significant increase in size perception of external objects. Results of the self and object size perception tests are in keeping with the hypothesis that the effect of LSD is to make thinking more primitive.

Dr. William Lahmon and his associates at Baylor University studied the effect of LSD on the perception of time. Whereas previous studies showed that the schizophrenic tends consistently to underestimate time periods, subjects under the influence of LSD showed increased variability in their time sense, both in the direction of underestimation and overestimation. Subjects reported an inability to make valid judgments of time. These differences suggest that the reaction of the normal to LSD produces a loss of temporal frame of reference, while the schizophrenics' consistent overestimation suggests a shift to a definite but invalid frame of reference. This finding shows an important qualitative difference, furthermore, between schizophrenia and the effect of LSD.

ELECTRICAL RESISTANCE OF THE SKIN IN SCHIZOPHRENIA

New studies of the electrophysiology of schizophrenia have been undertaken this year. In one such study, Dr. David Shakow and his associates at the NIMH compare the galvanic (electrical) skin response levels of normal and schizophrenic patients, the results of these comparisons with EEG (brainwave) patterns, and galvanic skin response in relation to certain drugs. Shakow found that normals, as compared with subjects who were not emotionally responsive, show greater response to galvanic stimuli. A chemical stimulant appeared to have little effect on responsivity,

but both a sedative and a tranquilizer lowered it. Most differences in reaction to the electrical stimulation of the skin were found to be related to certain EEG measurements. Patients with one type of EEG pattern were more responsive to tone than light, while another type of EEG pattern was found in patients who responded more to light than to tone.

EFFECTS OF BRAIN SURGERY

An NIMH grantee, Dr. Aaron Smith, in a follow-up study of brain-operated schizophrenics after an interval of eight years, found permanent losses in certain intellectual functions that are related to site of surgery and age of the subject.

Previous studies of the effects of psychosurgical techniques on mental functioning have measured psychological traits during the immediate postoperative period. Such studies have, in general, concluded that operations such as frontal lobotomies do not produce losses in mental abilities.

Dr. Smith retested 27 operates and 23 controls of a 1949 study of psychosurgical techniques eight years after their operations. Operates were classified as to age and site of the operation (i.e., superior or orbital topectomies of the frontal lobes). Contrary to other studies of the immediate postoperative period, Smith found that the Porteus maze test scores of 17 patients who received superior topectomies declined significantly below their original and immediately postoperative test scores.

This study points up not only the permanent losses that may be related to certain psychosurgical techniques, but also the importance of an adequate time-interval in studies of the effects of psychosurgery.

MECHANISM OF ELECTROSHOCK TREATMENT

For a number of years a group of investigators at the Hillside Hospital in New York have studied neurophysiological alterations occurring in electroshock treatment. Results of their experiments indicate that the behavioral changes evident in patients after electroshock therapy are the result of actual alteration in brain function induced by the electroshock process.

NIMH grantee Dr. Max Fink and his associates at Hillside Hospital, Glen Oaks, N.Y., compared the degree of clinical improvement in patients undergoing electroshock therapy with two indices of brain function: (1) the degree of induced delta activity in the

electroencephalogram, and (2) changes in orientation and awareness after amobarbital sodium medication. Those patients rated as most improved after a course of shock therapy were the same ones who showed the greatest increase in EEG delta activity and positive amobarbital tests. Equally significant was the cluster of negative amobarbital tests, low or moderate EEG delta activity, and clinical ratings of "unimproved" at the other end of the scale.

This finding indicating that behavior change correlates positively and significantly with alteration in brain function after electroshock therapy suggests that similar studies of other forms of somatic therapy for mental illness may yield results which illuminate the relationship between brain function and behavior.

In an attempt to unify these and other findings which relate to physiological therapies, Dr. Fink points out that shock, lobotomies, insulin treatment, and tranquilizers all produce a persistent change in cerebral function, of which shift in the delta rhythm on the EEG and increases in seizures are reflections. He suggests that these changes are not specific to any given disorder but that, by changing the way in which the brain functions, they produce behavior changes. These may then be evaluated as improvement or else provide a basis for behavioral readjustment.

STUDIES OF THE BIOCHEMICAL BASES OF BEHAVIOR

Studies of the biochemistry of behavior have moved forward along four pathways this year: studies of the metabolic fate of chemicals of known psychiatric significance, of the relationship of behavior to specific drugs, of the relationship of behavior to hormones, and synthesis of behaviorally significant chemicals. Increased understanding of the chemical structure, metabolic utilization, and behavioral significance of chemical substances is one of the basic foundations for understanding major disturbances of personality and is a prime source of hypotheses relating to the biology of such diseases as schizophrenia.

METABOLISM OF A POWERFUL STIMULANT

At the NIMH laboratories this year, Dr. J. Axelrod has described the metabolic pathways followed by epinephrine. This substance, in addition to its effects upon smooth or involuntary muscle, is related to a wide variety of emotional responses.

Its production in the body is increased by anxiety and it is known that increased production of this compound is part of the way in which the body mobilizes to meet both physical and emotional emergencies. A knowledge of how it is produced in the nervous system and what happens to it after it has been used is essential not only for an understanding of body chemistry, but also for possible medicinal utilization of either this chemical or an agent which counteracts it. As recently as 1956 very little was known about this substance (which is also known as adrenalin). It was known that only two percent of the epinephrine produced by the body is present in the urine. Because the mechanism by which epinephrine is destroyed in the body influences the rate of bodily activity, finding the enzyme which affects the process of destruction could prove a way of controlling levels of epinephrine in the body. It had previously been learned that monamine-oxidase was important in the destruction of epinephrine-like substances, by converting them to a chemical called VMA. However, only 40 percent of the adrenalin produced by the body or injected into it could be accounted for by VMA excretions. Dr. Axelrod, using adrenalin which had been "tagged" with radioactive tritium, found an intermediate step in the destruction of epinephrine. The bodily enzyme O-methyltransferase produces a change in adrenalin which converts it to a new compound, which Axelrod has named metanephrine. Metanephrine is then converted to VMA by monamine-oxidase. In urinalysis one finds two percent epinephrine, 50 percent metanephrine, and 40 percent VMA. Thus Axelrod has accounted for the metabolic fate of this powerful substance in the body, and described each of the chemical steps in its breakdown. This compound, in addition to being related to the behavior of the cardiovascular system, is related to anxiety, plays an important role in emergency reactions of the body, and, according to some theories, is intimately related to schizophrenia.

THE ACTION OF THYROXINE

Another major contribution in the understanding of a biochemical agent known to have major significance in behavior was reported by NIMH scientists Drs. Louis Sokoloff and Seymour Kaufman. Their work throws new light on the processes involved in growth, metamorphosis and development, and helps to explain many of the clinical features of abnormalities in hyperthyroidism. It also serves to explain the presence of retarded development of the body, and especially the brain, in cretinism.

It has long been known that thyroxine controls metabolism in some way. It had been thought that increases in thyroxine levels resulted in generally increased metabolic rates. Some six years

ago Sokoloff studied thyroid patients and found that while administration of thyroxine did increase metabolic activity of the rest of the body, the metabolism of the brain did not increase. This year Sokoloff proved that the reason that brain metabolism is not affected by thyroxine is that the brain does not use the excess protein produced, nor is it affected by changes in protein levels after the early years of brain growth have passed. These findings constitute a major contribution to an understanding of the mechanism of action of thyroxine in the body.

NARROWING ATTENTION

NIMH career investigators Dr. Enoch Callaway and Dr. R. I. Band at the University of Maryland analyzed evidence that emotional states and certain chemical agents can produce similar narrowing of attention. In further experiments this year they have found that atropine appears to influence behavior by broadening attention. In practical behavior situations, tasks which demand broad attention are assisted by atropine whereas tasks demanding a narrower focus of attention are hindered by this drug.

DRUGS AND LEARNING

Dr. Conan Kornetsky and his associates at NIMH, using normal young volunteer subjects, have been studying the behavioral effects of three widely used drugs--meprobamate (a tranquilizer), phenobarbital (a sedative), and dextro-amphetamine (a stimulant). Dr. Kornetsky has demonstrated that high dosages of the tranquilizer significantly impaired motor response, choice-reaction-time, and learning. Low dosages of the tranquilizer reduced the rate of learning, but did not affect motor response or choice-reaction-time. Subjecting his subjects to long periods of sleep deprivation, the experimenter found that the stimulant significantly improved performance on all three behavioral tests after 68 hours of sleeplessness, and in all tests except learning after 44 hours of sleeplessness. He observed that the dosages of the stimulant did not make the performance of tired subjects significantly superior to their performance in a nonfatigued state, but merely brought them back to approximately normal levels. Dr. Kornetsky also observed that, as the task required of the subject became more complex, the stimulant was less likely to restore normal functioning level.

DRUGS, SLEEP AND AROUSAL

Dr. Edward Evarts and his associates in the Section on Physiology at NIMH are attempting to understand some of the processes underlying behavior and perception by studying animals in whose brains electrodes have been permanently implanted.

This year their studies have focused upon the difference between sleep and arousal. Dr. Evarts found that, under conditions of normal sleep, the cortex was significantly more excitable than during the waking state. This relative increase in excitability during sleep may in part account for the forgetting of dreams and may explain why very small external stimuli can produce highly detailed dream material. Dr. Evarts studied the effects of certain drugs on this difference in the electrical activity in the brain. He found that an anesthetic agent and a barbiturate do not produce the same pattern of excitability as normal sleep. However, tranquilizers did produce a pattern which is indistinguishable from normal sleep activity. These findings, increasing our understanding of the physiology of sleep, may have application to many practical problems such as driver safety.

MOOD CYCLES AND DRUGS

A number of investigators have been working on the relationship of certain hormonal substances to human behavior. Dr. Curt Richter at Johns Hopkins University demonstrated the existence of certain regular cycles of mood and metabolism in both normal and disturbed people. In some instances catatonics with 20-to-40 day cycles have been helped by hormones. Richter has demonstrated that analogues of these human cycles can be produced in rats by interference with thyroid functioning. Since each of these cycles has a normal, as well as an abnormal phase, Richter suggests that the normal phase of a mood cycle can be used as a baseline for the study of abnormal behavioral and metabolic conditions in individuals.

STRESS AND ACTH

It has been widely suggested that the antidiuretic hormone (ADH), released from the central nervous system as a result of stress, triggers the release of adrenal corticotropic hormone (ACTH) from the anterior pituitary body and starts the complex chain of physiological reactions to stress. NIMH and NHI scientists have now demonstrated that there is no necessary or apparent relationship between ADH and ACTH release, and that the central nervous system effects the release of ACTH in some way as yet unknown.

NORCODEINE EFFECTS STUDIED

In a logical extension of investigations comparing the effects of morphine and normorphine, workers at the NIMH Addiction Research Center, Lexington, Kentucky, have performed a number of studies comparing the effects

of codeine and norcodeine. In general, norcodeine's action in relation to codeine's strongly resembles normorphine's action in relation to morphine's.

In direct addiction tests, tolerance to the sedative effects of norcodeine developed more slowly than did tolerance to the effects of codeine, so that after 18 days of addiction 400 mg. of norcodeine produced the same amount of sedation as almost 1500 mg. of codeine. The relatively slow rate at which the body develops tolerance to norcodeine means that patients can be maintained on much smaller doses of norcodeine than of codeine with equal effect.

Another desirable characteristic of norcodeine is that the abstinence symptoms which develop in addicted subjects upon abrupt discontinuance of the drug are much milder than the symptoms of withdrawal from codeine addiction. Of the four drugs--morphine, normorphine, codeine, and norcodeine--norcodeine has the lowest addiction liability and the mildest withdrawal symptoms.

Norcodeine proved to be effective in suppressing abstinence symptoms when it was substituted for morphine in morphine addicts. The clinical advantage of this substitution procedure is that withdrawal of addicts from norcodeine is easier for both patient and doctor than direct withdrawal from morphine.

These studies are part of the Addiction Research Center's continuing program of testing and evaluating addicting and potentially addicting drugs and seeking to develop drugs with high sedative and analgesic potency but low addictive liability.

PITUITARY REMOVAL AND PERSONALITY

Previous investigations of the pituitary gland have suggested a possible relationship between this gland and psychological states. Dr. Martha Schon of the Sloan-Kettering Institute this year studied the psychological states of a group of women from whom the pituitary body had been removed as part of the surgical halt of their cncerous breast conditions. She administered a battery of psychological tests both before and after the removal of the pituitary gland. While all the women showed some fear of death, facial disfiguration, and mental or personality impairment before the operation, all wanted to go through with it, and all tolerated it well.

None of the women showed significant impairment in intellectual functioning after the operation. Some personality changes, however, did appear. The women tended to be more flexible in their way of thinking, more easily affected by emotional stimulation, and less exact in their perception of their environments. In addition, there appeared to be a decrease in aggressiveness in interpersonal relationships, a decrease in depression, and a perceptible decrease in sexual interest. Some of the women also reported impairment in their ability to smell and to taste food. Other results of the operation include increased weakness and fatigue, sensitivity to low temperatures, and increased irritability. These side effects of surgery tended to disappear upon administration of thyroid substitute hormones.

NEW DRUG SYNTHESIS

Drs. David A. Shirley and Purnandu B. Talukdar (University of Tennessee) have successfully synthesized a number of dialkylamino-alkylabenso (a) phenothiazines which have been submitted to a drug company for pharmacologic testing. By substituting several different dialkylaminoalkyl groups on the nitrogen atom of the parent benzophenothiazine molecule, the investigators hope to have produced compounds with some chemotherapeutic activity.

GENETIC CHEMICALS

Ribonucleic (RNA) and desoxyribonucleic acid (DNA) have been the subject of much recent investigation. DNA, chiefly present in the nucleus of cells, appears to play a crucial role in passing genetic information on from generation to generation. RNA, chiefly found in the cytoplasm of cells, appears to have a fundamental part in the synthesis of protein. Since for both of these related substances the communication of chemical information seems to be a central function, an understanding of their structure and the way this structure operates to pass on information is essential. Because RNA is somewhat unstable chemically, it has been most fruitful for investigators to study its structure through use of synthetic polynucleotides, created by the conversion of nucleotide diphosphates. Dr. Alexander Rich, a scientist at NIMH, has now reported the discovery of a new two-stranded helical configuration involving polyadenylic acid and polyinosinic acid, and of a three-stranded configuration, in which a second polyinosinic acid strand is twined around in the helical groove of the original polyadenylic-polyinosinic acid chain.

The increased knowledge of the configuration and functioning of the synthetic polynucleotides thus afforded is another important step in the progress we are making toward a full understanding of the way two of the basic substances of life--DNA and RNA--carry

genetic information and dictate the patterns of protein synthesis. These discoveries help elucidate, at the most basic level, the functioning of the living nervous system as well as of all living matter.

CHEMICAL ADVANCES IN RETARDATION

Hundreds of newborn babies each year suffer almost total deprivation of intellectual capacities as a result of the hereditary ailment, phenylpyruvic oligophrenia; Dr. Seymour Kaufman's clinical studies at the NIMH Laboratory of Cellular Pharmacology have further illuminated the metabolic processes involved in the disease.

A more precise delineation of the metabolic block responsible for the disease has been made possible by purification from boiled rat liver extract of an organic nonprotein cofactor involved in the phenylalanine to tyrosine reaction. This more complete determination of the pathway may lead to better understanding of the relationship of phenylalanine and tyrosine metabolism to normal brain development and function.

BRAIN AND BEHAVIOR

A number of studies have been cited in which structural aspects of cerebral activity have been shown to play a significant role in behavior. Among the many studies concerned with neurostructural aspects of behavior have been investigations of the relationship of brain lesions to behavior, new techniques for studying specific portions of the brain, and the relationship of nervous system activity to various chemical agents.

Earlier in this report a study by Dr. Aaron Smith was described which reported changes in the intellectual functions of schizophrenics who had undergone brain surgery. Dr. H. E. Rosvold and his associates at the NIMH laboratories surgically produced lesions in portions of the brains of 23 monkeys. All subjects were then trained on a visual discrimination task and on a single alternation task. Visual discrimination was not impaired in any of the animals. Single alternation performance was impaired, and was related to lesions in three different portions of the brain. This interesting finding indicates very widespread anatomical participation of the brain in tasks involving even the slight complexity of the alternation task. This kind of study serves as a continual reminder that the possibility of finding simple specifically localized areas of the brain corresponding to complex behaviors is probably not reasonable.

A STUDY OF MEMORY

Studies of discrimination test performance of brain-operated monkeys have led to the formulation of new hypotheses concerning the operation of memory.

Some monkeys were given anterior frontal lesions, others inferior temporal lesions, and some remained unoperated in experiments designed by Dr. Mortimer Mishkin of the NIMH and Dr. Lawrence Weiskrantz of Cambridge University.

Results indicated that, when there is an abrupt increase in delay of reward, animals with frontal lesions performed more poorly than both the operated and unoperated controls. Delays within trials, separating either response and reward, or cue and response, may present difficulties for these animals. In contrast, on tests involving only delays between trials, animals with frontal lesions learn as rapidly as do unoperated controls and are even superior to the controls with temporal lesions.

This suggests that two distinct kinds of "memory" are at work in visual discrimination performance tests: a short-term, dynamic memory trace bridging delays when there is a separation of events within a trial; and a long-term, more permanent kind of memory trace which probably accounts for the cumulative effect of learning between trials.

NEW APPROACHES TO PARTS OF BRAIN

Drs. Richard E. Coggeshall and Paul D. MacLean of the NIMH Laboratory of Neurophysiology have succeeded in obtaining extensive hippocampal lesions in mice and rats through chronic dosage with 3-acetylpyridine (3AP). Complete surgical removal of the hippocampus as a means of testing hypotheses regarding its role had been found to be impossible without causing some damage to other structures.

Weakness of the extremities, impaired locomotion, inspiratory rhonchi, urinary incontinence, weight loss, and an unkempt appearance occurred in the animals after the drug-induced lesions. Histological examination showed slight to complete loss of neurons in the areas of the hippocampus designated CA3 and CA4 by Lorente de No, without significant damage to other parts. This study has added to the growing evidence that the metabolism of the hippocampus is distinctive from that of other types of cortex.

Still another approach to the study of localized brain function was reported by Drs. Bruno Minz and E. J. Walaszek at the University of Kansas Medical School. These investigators applied epinephrine directly to the exposed cerebral cortex of rabbits. It has been known that such topical application produces a rise in blood pressure. These NIMH grantees are studying the effect on this response of pretreatment of the animal with certain drugs and with serum drawn from schizophrenic patients and from normal controls. They have demonstrated that pretreatment with the tranquilizer, chlorpromazine, blocks it, indicating that these tranquilizers have different modes of action. They found further that schizophrenic serum blocks or reverses this elevation in blood pressure while normal serum appears to have no effect on it. This finding suggests the existence of an unknown factor in schizophrenic serum which is responsible for this difference in response.

In further experiments the workers found that the epinephrine and norepinephrine content is much higher in the brains of rabbits pretreated with schizophrenic serum than than in the brains of rabbits pretreated with normal serum. While these studies have not yet been confirmed, they point to a close linkage between physical function of the body, biochemical structure, and behavior.

LEARNING AND PERCEPTION

EARLY RADIATION AFFECTS LEARNING

NIMH Grantees Ernest Furchtgott, M. Echols, and J. W. Openshaw of the University of Tennessee, mass-tested groups of rats who had received systematically varied amounts of exposure to X-rays during the period before and immediately after birth. Their experiment demonstrated that learning was affected by the size of the dosage and the time at which the rat received the radiation. The greater the dosage, and the earlier in the rat's development that he receives it, the greater will be the later deficit in learning. The significance of this study is both evident and pressing.

AUTONOMIC LABILITY

Drs. John and Beatrice Lacey of the Fels Research Institute in Ohio, continuing their long-range research on the relationship of the autonomic nervous system to personality and learning, have confirmed that the rate of an individual's autonomic fluctuations, as measured by skin resistance and heart rate, is a reliable characteristic of that individual. Persons

who can be described by these techniques as autonomic "labiles" have been shown to have faster reaction times than autonomic "stabiles," and can maintain maximum preparation to respond longer than can the "stabiles." However, autonomic "labiles" are not able to restrain or inhibit their responses as well as can the autonomic "stabiles." In another study, Dr. Lacey showed how autonomic responses could be used to study unconscious anxiety, and to measure the readiness for anxiety in individuals.

ESTIMATING OBJECT SIZE

NIMH scientist, Dr. V. R. Carlson, has demonstrated that the tendency to overestimate the size of a distant object is related to the subject's expectations and motivation.

As you move away from an object, the image of the object on your retina becomes smaller. Despite this physical fact, you continue to "see" the object as having approximately its usual objective size. This phenomenon is known as size constancy. Actually, size tends progressively to be overestimated somewhat with increasing distance, a fact which has not been satisfactorily accounted for in any theory of perception.

Using instructions designed to eliminate certain biases usually present in this experimental situation, observers were asked to match a series of distant standard triangles with a variable near triangle. The experimental design allowed the subjects--but did not require them--to distinguish between apparent visual size and objective size. The subjects nonetheless did clearly produce different matches corresponding to these two concepts but not in the way usually assumed in terms of traditional perceptual theory. They overestimated size when trying to judge actual size, and achieved perfectly accurate matches (on the average) when trying to judge apparent visual size. This difference is interpreted as due to a shift in attitude on the subject's part rather than to a sensory stimulus-determined process. A significant correlation with intelligence test score further strengthens this argument. An additional experiment with subjects in differing motivational states suggests that the person's motivation to do well in the task (i.e., be accurate) leads him, unknowingly, to overcorrect for a presumed diminution of apparent size with distance. Dr. Carlson has not only provided an explanation of the classical problem presented by size overestimation, but has further demonstrated the potential utility of the phenomenon of size constancy as a tool for the study of such abnormal conditions as schizophrenia, which are characterized by perceptual disturbances.

FACTORS OF SENSORY DEPRIVATION

Drs. Milton Greenblatt, Henry V. Grunebaum, and Sanford J. Freedman (Harvard and Brandeis Universities) tested groups of normal subjects in two situations. The individuals were subjected to a marked reduction of sensory stimulation, or to social isolation. The range of responses to the sensory deprivation was very broad. Some people could not stand the situation, loss of time sense was a general distressing reaction, and the subjects showed considerable change both in their perception of simple figures and in the accuracy of their visual-motor coordination. These symptoms were not present among the socially isolated subjects. Subjects who were deprived of sensory stimulation also showed considerable distortion and difficulty with concentration, description of their body image, hallucinations, and fears.

The breakdown in perceptual functioning and the alteration of ego functioning which was observed may provide a clue to increased understanding of schizophrenic behavior, and may help to anticipate the behavior of normals who must operate in isolated situations, deprived of normal sensory and social stimulation. The symptoms observed, for example, are very similar to the symptoms of the LSD psychosis. Since the schizophrenic is often described as "withdrawing" or "isolating himself", it may be that the subjection of normal individuals to sensory and social isolation may be an improved way of producing a model psychosis for study and analysis, eliminating the toxicity problem inherent in the use of drugs.

Since many present and anticipated military and industrial assignments involve some sensory or social isolation, studies of this sort may help in planning ways of minimizing conditions which would hamper effective performance and techniques for selecting suitable personnel for such assignments.

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PSYCHOPHARMACOLOGY

In the application of drugs to the control of behavior, in the evaluation of the effectiveness of such drugs, and in understanding both the biological mechanisms of the drugs and their relationships to complex psychological processes, there is a testing ground for the theories and findings of scientists who work in both the behavioral and in the biological sciences. The continued growth of research in psychopharmacology is reflected in the wide variety of studies reported this year.

These studies announce new drugs and new techniques for testing them. Intensive studies of the effects of drugs on motivation, development, and behavior have been completed. An important new technique for pinpointing the action of drugs has been reported.

A PROMISING NEW DRUG FOR DEPRESSION

Imipramine may prove to be valuable as a safe, specific treatment for mental depression. NIMH grantees recently reported a preliminary trial in which 83.6 percent of 55 depressed psychotics and neurotics showed marked to moderate improvement following three to six months' treatment with imipramine. Side effects were minimal and were controlled by decreasing the dosage. Imipramine, which is structurally related to chlorpromazine, is N-(3'dimethyl-aminopropyl)-iminodibenzyl hydrochloride, a compound which has principally sedative effects in animals. Its antidepressant effects were noted in 1957 by R. Kuhn of Switzerland.

Dr. H. Azima and Dr. R. H. Vispo, of Allen Memorial Institute in Montreal, conducted a trial in which 55 depressed psychotics and neurotics were treated with oral doses of 50 to 200 mg. imipramine per day for 3 to 6 months. (The drug was stopped for patients who failed to respond favorably within three weeks.) Of the depressed patients, 43.6 percent improved markedly and 40 percent moderately; three patients showed slight improvement, and only six failed to improve at all. When the drug was withdrawn after four to eight weeks' treatment, symptoms of depression recurred within a week. The eight nondepressed patients who were tried on the drug showed no response.

These findings suggest that imipramine has a specific effect on symptoms of depression. Anxiety not related to depression was not affected. Further, imipramine appears to be a safe drug. Side effects were minimal and were controlled by reducing the dosage. No adverse effects were seen on the liver function tests, white blood counts, differential counts, urinalyses, blood pres-

sure measurements, or TPR, which were repeated regularly throughout the treatment period. A further point of interest is the contrast between oral and intravenous administration. When 25 to 50 mg. were intravenously administered, either no effect was seen or the drug merely sedated the patients.

These findings are encouraging, but will have to be confirmed by carefully controlled studies before the drug can be accepted as a specific treatment for depression.

NEW TESTS FOR TRANQUILIZERS

The past year saw continuing research on techniques for testing psychopharmacological agents. Because of the complexity of psychological processes the techniques used for testing other kinds of medicinal substances are not applicable to these drugs. Accompanying the development of new tests has been the development of new training programs to teach people how to do this kind of research.

Many tests of the psychological effects of tranquilizing drugs on experimental animals have measured the influence of the drug on behavior prompted by a stimulus related to punishment. For example, the slowing of an avoidance response to electric shock might be taken as a measure of the effect of a particular drug. Such tests measure drug effects in terms of decrements in behavior. Dr. D. S. Blough, while working at NIMH, developed a test which is not associated with anxiety or fear, and which is expressed in improved rather than impaired performance.

The test measures the ability of the experimental animal, in this case a pigeon, to stand still in a particular spot. The longer the animal stands still, the more likely it is to receive food reinforcement automatically through action of a photoelectric mechanism triggered by the shadow of the motionless bird's head.

To evaluate the new test, Dr. Blough compared the performance of birds after low doses of chlorpromazine and pentobarbital. On earlier conditioned avoidance and discrimination tests, these two drugs had produced similar results. On the new test, the drugs produced very different results. Chlorpromazine enabled the pigeons to stand still for a longer time than in control sessions, while pentobarbital significantly interfered with the ability to stand still.

It is hoped that this new test will prove to be useful in distinguishing other kinds of responses to a variety of drugs. If so, it may be a useful complement to the many tests of psychological response based upon behavior associated with anxiety or fear.

A TECHNIQUE FOR MEASURING THE EFFICACY OF PSYCHOPHARMACOLOGIC AGENTS

NIMH grantee, Dr. Max Fink of Hillside Hospital, Glen Oaks, Long Island, correlated drug-induced changes in behavior with EEG patterns in a study of acute and chronic administration of 19 psychotropic drugs (tranquilizers, energizers, psychotomimetics, and others) in hospitalized psychotics and psychoneurotics. EEG recordings were made before, during, and after the course of drug therapy. Behavioral evaluations were based on clinical descriptions by the patient, the physician, and the technicians, and on analyses of changes in language patterns as revealed in a tape-recorded, unstructured psychiatric interview.

The drugs which increased synchrony and slowed frequency in the EEG were associated with behavioral sedation, tranquility, and minimization of symptoms. An increase in synchrony and in frequency was associated with sedation, euphoria, hypomania, and decreased somatization. Desynchronization of frequencies was accompanied by increasing agitation, excitement, somatization, illusory phenomena, and hallucinatory and delusional ideation.

FIRST COURSE ON TECHNIQUES OF DRUG RESEARCH

Research being carried out by Dr. Peter B. Dews, NIMH grantee at Harvard University, has the dual purpose of investigating the behavioral effects of drugs on animals and training medical students in the methods used for such studies. Drs. Dews and W. H. Morse have published a detailed account of an experiment in psychopharmacology which is suitable for inclusion in a regular series of student laboratory exercises in pharmacology. Publication of their paper in the Journal of Medical Education may help to emphasize the importance of a knowledge of psychopharmacology for the practice of medicine and may encourage departments of pharmacology in other universities to include psychological research techniques in their laboratory training courses.

MOTIVATION AND LEARNING

Dr. Neal Miller and his associates at Yale University have been experimenting with a variety of ingenious surgical, neuroelectrical, and biochemical approaches and have been exploring the separate components of motivation, learning, avoidance, and conflict. In an earlier study he showed that stimulation of a certain portion of the brain of a rat produced behavior of a paradoxical sort. When an electrode was located in that

portion of the brain, and the experimental arrangements were such that the rat could stimulate himself, he alternately would turn the stimulation on and off. It was as though stimulation to this one place both rewarded and punished him. Research has further indicated that the reward-aversion responses were affected in different ways by tranquilizers and stimulators. The reward component seems more susceptible to low doses of these drugs. Where the stimulant increases the speed of turning the stimulus on, a tranquilizer decreased this activity and increased the speed with which the stimulus was turned off. This striking difference in response suggests that this technique may be useful in analyzing drug effects on different motivational systems in the brain. The motivation underlying mental illness is more important than the symptoms, for often one symptom may disappear or be alleviated only to be replaced by another.

DRUGS AND BEHAVIOR

A number of studies have been reported this year on the relationship of specific drugs to certain aspects of development and behavior.

DRUGS AND SOCIAL BEHAVIOR

Drs. Charles Savage and Juliana Day (NIMH) placed four chronic regressed schizophrenic patients on a two-week period of control injections, after which they were given 5 mg. daily of reserpine intramuscularly for a 30-day period. This was followed by another two-week control period, and so on for six months, at the conclusion of which the reserpine was stopped for a month. Recordings of therapeutic interviews were made and observations conducted daily.

The patients who improved showed an increased friendliness and a greater concern for the feelings of others. They also eagerly sought out old friends but continued to feel hostile toward strangers. While they were not any less aggressive, their behavior was nevertheless better controlled and more acceptable.

The process of psychotherapy was not enhanced, however, largely because the action of reserpine seems to lead to repression rather than expression of conflicts within the individual.

PSYCHODYNAMIC CHANGES

Studying the concrete personality changes of schizophrenics treated with tranquilizers, Dr. R. A. Cleghorn (McGill University) found a basic shift in patients' moods.

Using projective techniques plus the content of psychoanalytic interviews, Dr. Cleghorn reports that his patients partially abandoned their symptoms of withdrawal and splitting, and shifted from paranoid-like behavior to manic-depressive conditions. Their moods shifted toward elation and their experiental state moved towards feelings of greater wholeness.

DRUG SHOWS NO DEVELOPMENTAL EFFECTS

Dr. John L. Fuller, NIMH grantee at the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine, has reported that early results of experimental studies suggest that large doses of chlorpromazine have no adverse effects upon psychological development in young puppies. Although much additional work will have to be completed before definite conclusions can be drawn, these preliminary findings are reassuring in implying that chlorpromazine may be used without the risk of long-term effects on learning ability or other psychological functions.

The effects of chlorpromazine are being investigated in four groups of beagle puppies. The groups are treated alike up to seven weeks of age. For the next eight weeks, two groups are given chlorpromazine and two groups no drug. During this time each group is observed in a free environment, and in an environment in which infraction of certain "rules" is punished. So far, the results indicate that chlorpromazine did not interfere with learning in either situation; that puppies given drugs during the punishment period showed less after-effect of punishment; that sexual and aggressive behavior continued to occur in the drug-treated group but was diminished, probably because motor activity was reduced and performance was therefore impaired; that the effects of chlorpromazine varied according to the situation in which the puppies were placed. When a drug-treated and a no-drug puppy were playing together it was hardly possible to distinguish between them, but when each was playing alone the no-drug puppy was much more alert and active.

Following the 8-week period on chlorpromazine, no deleterious effects upon psychological development were observed. After-effects were either absent or benign in nature.

NEW TECHNIQUE PINPOINTS DRUG ACTION

In research supported by NIMH, Dr. James Olds of the University of Michigan is using an ingenious technique which permits rats to inject themselves with drugs. By means of a small tube inserted through the skull and permanently fixed to a particular

part of the brain, a rat can press a lever and have a drug injected directly into its brain. Findings from several studies emphasize the value of this technique as a method of determining at a very early stage which new drugs have behavorial effects that may be therapeutically beneficial to specific types of mental patients. The work may have important implications for the treatment of mental disease, and may also contribute to better understanding of brain function and the cause of mental disease.

The self-injection technique is similar to the technique that Dr. Olds and Dr. Peter Milner devised for electric self-stimulation of the brain. With the latter method, electrodes are permanently implanted in the rat's brain; the rat can stimulate the brain by pressing a lever which causes an electric current to be supplied to the electrodes. Through studies of electric stimulation, it was established that excitation or stimulation of a particular area of the hypothalamus was rewarding or pleasurable. Subsequently, pipettes or small tubes were inserted and fixed to the same area of the hypothalamus, and the rats were permitted to inject themselves with one of eight different solutions--seven drugs and physiological saline. The eight solutions produced different rates of response. Iproniazid produced the highest rate--over 300 self-injections an hour--far above the chance rate which was obtained with the saline. The "pleasurable" effects of iproniazid were so great that hungry rats given the choice of food or drug chose drug. This work suggests that the area of the brain that was most responsive to iproniazid, a drug which is used to treat depressed mental patients, is perhaps the same part of the brain that is involved in mental depression.

A second study was carried out in which the pipettes were fixed to 12 different points along the floor of the hypothalamus and telencephalon in 12 different rats. The animals were then trained to press a lever for iproniazid injection. The rates of self-injection varied according to placement of the pipette, indicating that the 12 points of the brain being stimulated produced different degrees of pleasure. When the injector was turned off, the rats continued to press the lever for 30 to 45 minutes and then stopped. When the injector was turned on again, the lever pressing quickly increased again to rates of about 300 self-injections an hour, thus further confirming that the high rates did not occur by chance. These techniques are being used in studies of tranquilizers, energizers, and a number of other drugs which produce changes in behavior.

INTERACTION OF DRUG AND ENVIRONMENT STUDIES

Dr. Peter B. Dews, NIMH grantee at Harvard University, has reported research findings which show that the behavioral effects of drugs may not be due solely to the drug itself but may be caused by interaction between environmental stimuli and drug. Using the operant-conditioning techniques of psychology, Dr. Dews, a pharmacologist, is studying the effects of various drugs on the behavior of pigeons and is attempting to isolate environmental factors which modify the behavioral effects of drugs. This kind of research may help to explain the variability of drug action and thus lead to more accurate prediction of drug effects.

Dr. Dews is using an apparatus in which pigeons are periodically given food rewards for pecking keys which are illuminated by different colored lights. The reward is given after a fixed number of "correct" pecks, or a fixed interval of time, or a combination of the two. In studying the effects of drugs on the performance of pigeons in such an apparatus, Dr. Dews demonstrated that the production of changes in behavioral output was related to the experimental environment of the animal and not to the dosage of chlorpromazine the animal had received.

Additional investigations point to an interesting relationship between the action of chlorpromazine and the effect of environmental stimuli on behavior. Dr. Dews has suggested that when an environmental stimulus tends to decrease output of behavior, chlorpromazine increases the over-all rate of responding; and, conversely, when the stimulus tends to cause a high rate of responding, chlorpromazine decreases over-all output. This hypothesis is being tested further; if proved, it will have important implications for the clinical use of chlorpromazine and other drugs.

NIMH grantee Dr. Granville Tolley at the University of North Carolina has been conducting systematic physiological and psychometric studies of tranquilized and non-tranquilized patients. While he has found many of the same differences between drug and control groups which have been reported elsewhere--changes in tests, changes in the reports of psychotherapists and residents, etc.--these differences do not appear in the reports of ward behavior made by nurses and attendants. These ward personnel are not aware of which patients are receiving the drugs and which are receiving tranquilizers. Currently Dr. Tolley is making a detailed study of the behavioral check list used on the wards, but the data so far indicate that the improvement in ward behavior shown by both groups of patients reflects the increased expectation by the ward staff that their behavior should improve.

DISCHARGES, DRUGS, AND OPTIMISM

Dr. Erwin L. Linn of the NIMH staff, studying a group of patients first admitted to Saint Elizabeths Hospital from 1953 through the first half of 1956, found that patients whose first admission to the hospital came since the introduction of tranquilizing drugs were more likely to be discharged from the hospital than patients who entered before the introduction of the drugs. This was true whether or not the patients actually received drugs. Furthermore, he showed that the rate of discharge was the same, since the beginning of drug use, for patients who received drugs as for patients who did not receive tranquilizers. Whereas it had been maintained that the drugs were particularly effective with overactive patients, Dr. Linn demonstrated that the rate of discharge was the same for overactive and for passive and withdrawn patients. Dr. Linn found, on the other hand, that since the drugs were introduced the nurses, attendants and doctors at the hospital had become more optimistic about the patients' chance of recovery and that this new hope on their part produced changes in the emotional climate of the hospital wards. Dr. Linn postulates that it is this change in social environment of the hospital that was the effective factor in the increased discharge rates.

Dr. George Brooks of Vermont State Hospital, working under an NIMH grant, has shown that chronic schizophrenics, who had remained too apathetic and inert under proclorperazine therapy to participate in or benefit from an intensive rehabilitation program, were sufficiently improved under a new phenothiazine, trifluoperazine, to participate actively in their own rehabilitation. Eleven of the fifteen chronic patients improved strikingly on the new drug, and seven became well enough to be ready for release to outpatient status.

EPIDEMIOLOGICAL STUDIES

The numerical drop in hospital populations, and the increase in net releases of patients since 1955 is statistically significant, even though admission rates have increased. Many people have ascribed this drop to the introduction of tranquilizing drugs.

DRUGS AND DISCHARGE RATES

Careful investigation of the statistics before and after 1955 by NIMH biometrician Dr. Morton Kramer indicates that the available data are inadequate to tell whether or not the introduction of drugs was a major factor in this change. State by State examination indicates that those

States which had been reporting lower mental hospital populations prior to 1955 continued their downward trend, while, in some instances where drugs are widely used, populations have continued to increase.

MENTAL ILLNESS RATES

It frequently has been stated that the increased numbers of mental hospital patients simply reflect better diagnostic procedures, greater numbers of hospital beds or the increased life expectancy of our population, and not a real increase in the proportion of mentally ill in the population. Certainly, as long as hospital admission rates exceeded discharge rates, institutional statistics could show increased patient populations even if, in fact, the proportion of mentally ill in the total population was actually decreasing.

Using data from the New York State hospital system where detailed records of first admissions have been kept for many years, and where State laws have required admissions even when bed space was not optimal, NIMH grantee Dr. Benjamin Malzberg (Research Foundation for Mental Hygiene) found that except for general paresis (whose decline has paralleled the gradual disappearance of syphilis), rates of admissions have increased in all categories of mental illness, and uniformly for all ages. Indeed, the chances of recovery for senile admissions have declined somewhat. Malzberg's data indicate that the increase in admissions is not simply a reflection of the increased age of our population, or the increased number of hospitals, or the diagnostic improvements of the last decades. Careful study of the rates of first admissions since 1920 indicates simply that there have been increased numbers of hospitalized mentally ill people in the New York population, due to factors as yet unknown.

ALTERNATIVES TO NEW HOSPITAL CONSTRUCTION

Because construction of hospital facilities and training of professional personnel have not kept pace with increasing demands for services, a number of investigators have been exploring problems in the architecture of hospitals, alternatives to hospitalization, and new modes of diagnosis.

NIMH-supported studies by Dr. Lucy Ozarin have suggested practical ways of making more efficient use of ward space and have recommended increased use of open wards. Evaluation of the "day hospital", in which patients live at home but come to the hospital for daily treatment, indicate that, in certain circumstances, it may be a practical alternative to costly construction.

ECONOMICS AND MENTAL ILLNESS

A recently published study suggests that the costs of mental illness, both direct and indirect, may exceed two billion dollars annually. Economist Rashi Fein (University of North Carolina), author of the second volume of the final report of the Joint Commission on Mental Illness and Health, estimates the cost of mental illness at 1.7 billion dollars a year plus an indirect cost of 325,000 labor force years, whose value he estimates at 728 million dollars.

FOLLOW-UP OF DISCHARGED PATIENTS

NIMH's Mental Health Study Center has just completed a follow-up study of discharged mental patients and found that, despite the still existing strong stigma attached to mental illness, very few of them reported any social rejection because of their previous hospitalization. They seem to have been able to re-establish old friendships, and many of them were able to resume participation in a wide range of community organizations.

BEHAVIORAL STUDIES OF SCHIZOPHRENIA

Paralleling the widespread investigation of biological, biochemical and neurological factors in mental illness are an increasing number of investigations of the social and psychological origins of these disorders.

This year saw the completion of a number of NIMH supported studies of the social origins of schizophrenia.

SOCIO-ECONOMIC STATUS AND SCHIZOPHRENIA

The work of Drs. Frederick C. Redlich and August B. Hollingshead at Yale indicates that in New Haven people in the lower socio-economic classes tend to receive the diagnostic label "schizophrenia" more frequently than others, and tend to be hospitalized more frequently than are people in the higher socio-economic levels.

In another study, Drs. John A. Clausen and Melvin L. Kohn of NIMH, studying the community of Hagerstown, Maryland, found no relationship between schizophrenia and socio-economic levels. Looking at case history material of schizophrenia patients, they found that only a small percentage of the patients had been social isolates in their early teens, and that two-thirds had had normal patterns of social behavior. While people from the higher socio-economic levels had shorter hospital stays, this seemed to be more directly related to the ability of their families to keep them at home than to any differences in the severity of their disturbance.

FAMILIES OF SCHIZOPHRENICS STUDIED

While it has long been recognized that the family must play some role in the production of this abnormal behavior, it is only recently that intensive studies of families of schizophrenics have been undertaken. NIMH grantee Dr. Theodore Lidz and his associates at Yale University have been studying family members and family structures in which schizophrenics were raised. In this study not one of the patients' families was reasonably well integrated. The mother's attitude toward the child generally reflected her attitude toward the marriage, and all of the marriages were gravely disturbed. Marriages were either torn by conflict or distorted by the necessity of one spouse passively to accept the pathological behavior of the other. Sixty percent of these patients had at least one parent who was a schizophrenic, a borderline schizophrenic, or paranoid. These parents literally trained their children

to be irrational. They surrounded the growing child with unreal concepts of life, and thus, when faced with conflict themselves, the children came to terms with life by misperceiving and distorting it.

Another study of the families of schizophrenics is being conducted by Dr. Don D. Jackson and his associates of Palo Alto, California. As a preliminary observation, Dr. Jackson has reported that parents with whom he has worked tend to make their children extremely dependent upon them and at the same time deny that the dependent responses of the child are appropriate to their relationship. Indeed, these parents threaten withdrawal of support if the child even tries to point out that the dependency exists. Like Dr. Lidz, he has found that mothers of these patients tend to reflect in the rearing of their children disturbed attitudes toward their own marriages.

PERSONALITY DEVELOPMENT

SOCIAL "IMPRINTING" IN INFANCY

The importance of early experience in determining adult behavior has long been recognized and, indeed, is a basic tenet of the whole field of psychoanalytical psychology. In a widely published study in this area, NIMH grantee Dr. Eckhard Hess of the University of Chicago, following Dr. K. Z. Lorenz' earlier work, has demonstrated a unique phenomenon which he calls "imprinting". A mallard duck, when exposed to a social stimulus during a period from 13 to 16 hours after birth, becomes permanently, and Hess finds, irreversibly conditioned to this stimulus. Hess' ducks are kept isolated from all visible stimulus from birth until the imprinting period has arrived. When exposed to a moving and "quacking" decoy as their first visual social stimulus, they thereupon act toward that decoy as though it were their mother. If their first social experience during this period is with a human being they immediately start following that human being and continue to act toward him as baby ducks act toward their natural mothers. These findings have been confirmed in studies of birds, insects, fish and various mammals, and by other investigators. If parallels to "imprinting" can be found in human infant development, they would have profound implications for child-rearing practices.

CONGENITAL PERSONALITY TRAITS IDENTIFIED

Through the intensive use of modern recording techniques, along with careful statistical and psychometric investigation, Dr. Richard Bell in the NIMH laboratories has been studying the appearance of personality traits in human infants immediately after birth and before exposure to maternal care. The data indicate

that it is possible reliably to identify six such traits--sensitivity to touch, response to arousal, intensity of mouth activity, distractability, muscle tonus, and rate of maturation. These studies are significant attempts to understand the behavioral propensities with which children are born. Recognizing the genetic and physiological components of emotional disturbances, this kind of study may provide a method of identifying the constitutional prerequisites of both disturbed and normal behavior.

CHANGES IN I.Q. WITH AGE

The studies reported above are concerned with the "fixed equipment" with which the organism reaches maturity. Other studies are concerned with propensities for change. NIMH grantees at the Fels Research Institute have studied I.Q. changes in 140 children who were tested regularly from infancy to age 10. They found that I.Q.'s do change, that these changes are related to certain personality variables, and that the direction of change can be predicted from observation of particular personality characteristics. By and large, children who are concerned with achievement, who are aggressive and competitive, and who are curious about the world around them, tend also to show increases in I.Q. Children who are passive and unusually conforming tend to show decreases in I.Q. The closer the genetic relationship of the children, the more similar the pattern of I.Q. changes will be, and these personality differences may be nonverbal ways in which intelligence manifests itself. The authors could find no relationship between rates of physical growth and mental growth during the first ten years of life. The indication that personality characteristics may affect intellectual development has significance for our understanding of child-rearing techniques, school programs, and the prediction of adult intelligence.

SOCIAL DEPRIVATION AND ADULT APPROVAL

Showing the sensitivity of the organism to environmental stimulation, Dr. Jacob Gewirtz at the NIMH laboratories has been investigating the relationship between the amount of social stimulation, the appearance of social behavior, and the effect of adult approval on children's behavior. Studying first and second grade children, Gewirtz exposed them to conditions in which some had a 20-minute period of isolation from social contact. Another group spent 20 minutes in a relatively neutral situation, and a third group was given considerable stimulation, before each group was exposed to a standard setting in which their social behavior and response to adult approval could be observed. Gewirtz found that social approval was most effective as a reward to the child after the brief period of isolation and the level of social activity was greatest after

such isolation. On the other hand, approval was least effective and social activity was least discernible after the period of high stimulation. This study demonstrates not only the need that children seem to have for social experience, but also points up the fact that even relatively minor changes in the amount of social stimulation can produce clearly observable changes in children's activities.

THE CHILD'S RELATIONSHIP TO HIS FAMILY

Research in family-child relationships among normal children is important for an understanding of what happens in illness as well as for understanding normal behavior. This year NIMH research in this field centered on three major problems: the development of techniques for measuring parental attitudes, the study of social class differences in parental behavior, and studies of the relationship of maternal behavior to the subsequent behavior of normal children.

PARENTAL ATTITUDE SCALE

Drs. Earl Schaefer and Richard Bell (NIMH) this year published a "Parental Attitude Research Instrument," a comprehensive scale which can be used to evaluate objectively parental attitudes toward child rearing and family life. This instrument has already proved useful to investigators in this and other countries, and the large number of investigations being carried out with this device will make it possible, for the first time, to correlate findings from many widely separated laboratories dealing with problems of parental attitudes. Furthermore, there already is considerable evidence in its first year of publication to indicate that the very existence of this useful instrument will stimulate a considerable amount of research in this crucial area. In the field of personality development this instrument may prove to have the same significance that the first standard personality tests had for the field of clinical psychology.

MEASURES OF MATERNAL BEHAVIOR

Among the results emerging from the Section on Child Development at NIMH is a new research instrument for the measurement of maternal behavior which has been applied to detailed behavior records of children who had been studied from the age of one month through the age of 14 years. This year's work indicates that much maternal behavior can be understood as falling along two major dimensions. The first of these refers to the extent to which the mother controls the child or allows him to make his own choices. The second dimension is one of hostility versus love.

Comparing mothers' behavior patterns with their children's behavior patterns, the investigators found that up to the age of three the behavior patterns of boys and girls were similar to those of their mothers. After the age of three, the behavior of boys more closely paralleled their mothers' behavior, whereas girls started to become more and more different from their mothers. They found that there is little relationship between the behavior of boys under three years of age and these same boys' behavior when they become older than nine. Studying the early factors which seem related to the child's later adjustment, the investigators found that a combination of the mothers' behavior patterns and the family's socio-economic status was highly predictive of later adjustment. While intellectual growth of boys was shown to be closely related to maternal behavior, this relationship was not found for girls. The intellectual growth of both boys and girls is related to their families' socio-economic status. Contradicting a popularly held notion about the bright child, these investigators found that the personality traits most closely related to high I.Q.'s in children were friendliness, cooperativeness, and attentiveness. Drs. Nancy Bayley and Earl S. Schaefer found that mothers of higher socio-economic status tend to be warmer and more accepting of their children, and that mothers of lower socio-economic status tend to be more controlling and punitive. For all mothers, these traits were more consistently expressed toward their sons than toward their daughters, with the interesting exception that the younger boys from the higher socio-economic levels and younger girls from the lower socio-economic levels tended to be allowed more freedom than their brothers and sisters.

SOCIAL CLASS AND PARENTAL AUTHORITY

A study of the relationship between social class and parental authority was completed this year by Dr. Melvin Kohn of NIMH's Section on Community and Population Studies. Because of its immediate interest for a wide number of community problems, it is presented in some detail here.

Using census and housing tracts, the investigator selected 200 families of "white collar workers" and 200 families in which the father was engaged in a manual occupation; in each case, the family included a child enrolled in the fifth grade in a public or parochial school. In every family the mother was interviewed, and in every fourth family the father and the fifth-grade child were interviewed. The interviews concentrated on the ways in which mothers and fathers exercised authority and disciplined their children, and the kinds of situations which brought about disciplinary action. Considerable differences were found between middle and working class families, and between maternal and paternal attitudes toward discipline. Among working class mothers, children

were punished primarily for wild play and for loss of temper. Middle class children were punished for refusing to obey. While loss of self-control is not tolerable to the working class mother, she does not get upset when her son fights with friends and neighbors. In general, the working class mother punishes her child when the child does something which immediately interferes with her, rather than in terms of the seriousness of the child's misconduct. Middle class mothers tend to base their decision to punish on the basis of their interpretation of the child's intentions rather than actual behavior. For example, whereas the working class mother will almost always punish the child for doing something which interferes with her work or her comfort, the middle class mother will only punish such behavior if the child's intention is malicious. The working class father is less likely to punish wild play than is his wife. In general, the working class father tends to ignore behavior that does not interfere with his own comfort. The middle class father is more concerned with the long-range meaning of behavior to his child's future happiness; he will tolerate behavior that he sees as having long-range usefulness and punish behavior that he sees as damaging to the child's character. Middle class fathers furthermore tend to punish physically all fighting whereas the working class father is more tolerant of this behavior.

Summarizing these findings, the data indicate that the working class parent responds to action, the middle class parent to intention; the working class is concerned with obedience, the middle class with the child's happiness and self-control. For the working class parent respectability is a major parental goal for the child, whereas in the middle class, happiness is the major goal for the child.

THE DISTURBED CHILD

The increasing numbers of unmanageably hostile children, the shortage of trained personnel, the need for more efficient treatment programs, and the need for information on the most effective means of operating treatment centers, have made research on aggressive children of considerable practical importance.

NEW INTERVIEW TECHNIQUE

During the past year, investigators in the NIMH Child Research Branch have succeeded in delineating the structure and content of the "Life Space Interview". This technique is a new approach to psychotherapeutic interviewing, the "Life Space Interview" is focused on particular events in the child's experience and is conducted by a person whom

the child perceives as part of his everyday life. Its goals are the clinical exploitation of life events and the administration of emotional first aid on the spot. The strategies of the technique involve the selection of themes which the child sees as immediately relevant to his own life; issues which are clearly presented by a person who is close to him; the selection of such roles by the therapist as are compatible to the child's condition; the direct management of the child's moods; the use of timing as a therapeutic variable; and the use of the child's own setting as an aid to therapeutic impact. Contents of a "Life Space Interview" may include play, interviewing, control, reassurance, helping the child face reality, knowing when to be inactive, and interpretation. This technique is important not only as a tool for dealing with the emotionally disturbed and delinquent child, but also has direct application to the everyday problems of disciplining normal children. Dr. Fritz Redl has demonstrated here that the time to deal with youngsters' disciplinary problems is at the time and place that they occur.

ORIGINS OF AGGRESSIVE BEHAVIOR

Why does a child act in a hostile and hyperaggressive manner? The family studies cited above may find some answers to this question. In examining the life histories of normal and "acting out" children, Drs. Harold Raush and Allen Dittmann at the National Institute of Mental Health find evidence that the interaction of a hyperactive infant with an initially loving parent who later rejects the child's mobility is a likely precursor of "acting out" behavior. These are not consciously rejected children, although the parents may become desperate and finally develop secondary rejection. Generally the parents seem to be about as mixed up as the children, and the child's brothers and sisters also tend to be disturbed.

DELINQUENT PERSONALITIES

Drs. Robert D. Wirt and Peter F. Briggs at the University of Minnesota this year published results of a study of personality and environmental factors in the development of delinquency. The authors, using a novel experimental design, were able to identify personality types which were distinctive for delinquents. They then obtained large samples of boys of each of these types, and selected two other groups of boys whom they matched with the delinquents in terms of personality patterns. In brief, they assembled two pairs of groups. In each pair the boys were homogeneous as far as personality patterns were concerned, but in each pair one group was delinquent and the other was not. They then obtained detailed case histories, and examined the records of community agencies in

the areas where the boys lived. Some findings are summarized below: (1) 42 percent of the delinquents' families were known to social agencies prior to the child's delinquency. This was true for only 23 percent of the nondelinquents. (2) The severity of the delinquents' behavior was most closely related to marital disruption, and social behavior on the part of the parents. In addition, the parents of delinquent children were out of the home more frequently than was true for the nondelinquent group. In the course of gathering the histories, it became apparent that family conflict was found more usually in the delinquent home, and that the mothers of delinquents tended to give a rosier picture of their homes and to disagree more sharply with their sons' descriptions of family life. An interesting fact was that while many of these families had gone to social agencies for help with social and financial problems prior to the delinquency, none of them had received counseling focused on the emotional problems connected with the children's delinquent behavior. The authors point to the frequent lack of coordination among agencies, and the prevalence of highly specialized agencies that characterize most communities as the cause of this lack of potential preventive service. In addition, activities such as scouting and community recreation centers which are voluntary and depend upon self-selection often fail to attract the potentially delinquent youngster. Most of the delinquent children had not participated in any of the activities which many communities provide in the hope of reducing delinquency.

Delinquency was found to be an incidental result of personality structure. The investigators point out, however, that the typical personality characteristics of these youngsters--sensuality, superficiality, and self indulgence--are not enough to produce delinquency, but require, in addition, a poor and neglectful home. The personality difficulties these youngsters display are character disorders, only 7 percent of the group were classified as neurotic.

HELPING DISTURBED CHILDREN LEARN

One of the practical questions which has been asked is whether or not the specialized environment of a children's institution for hyperaggressive youngsters can assist these youngsters in deriving satisfactory learning experiences from school. The staff of the Child Research Branch has found that by constructing special, emotionally neutral curriculum materials, satisfactory school progress can occur. While for normal children, the use of learning content which is familiar to the child's experience facilitates his learning, these same materials discourage learning when presented to a child whose everyday life produced such severe

hostility that his removal from the community was required. Awareness of this simple fact, together with the development of specialized learning material for these youngsters, will have immediate practical consequence for the many children's treatment programs now being conducted all over the country.

RETARDATION

This year has marked the beginnings of many studies in the field of retardation. Most of these are still too new to have reported results. Some work, however, has been brought to completion. Drs. Roland G. Westall and Sheldon Miller and their associates at New York University have found a new kind of metabolic disturbance in which a deficit of amino acids in the body produces "maple sugar urine disease". This disease is characterized by early mental deterioration, and the investigators hope to develop a synthetic amino-acid diet to combat the disease.

During the past year Dr. Morton Kramer and his associates in the National Institute of Mental Health have devised a method for determining probabilities of stay, release, and death for patients admitted to a hospital for the mentally deficient. They demonstrated the use of this technique with data obtained from Pacific State Hospital in California for the years 1948 to 1952. The possibilities of getting out of the hospital were shown to be lowest for males and for those with mental deficiencies due to traumatic or infectious processes; and highest for teen-agers and for patients with I.Q.'s of 50 or over. The probability of death was highest for the youngest patients, the Mongoloids, those with cranial anomalies, and those with the lowest I.Q.'s. The method used in this study has obvious practical significance for educational, administrative, and treatment programs.

ALCOHOLISM

This year saw the initiation of a number of research grants in alcoholism. Several of the current projects are concerned with basic metabolic factors in alcoholism; several are attempting to improve treatment methods; and still others are studying the effect of alcoholism on physical health, intellectual functioning, and personality.

NUTRITION AND ALCOHOL

Dr. Maurice Victor (Harvard) reported recently on the nutritional diseases of the nervous system that are associated with alcoholism. In these diseases alcohol produces its damaging effect by displacing food in the diet. In his study

of 131 patients suffering from these diseases, Dr. Victor found what he terms "fantastic reduction in food intake for long periods." The specified nutritional factor responsible for such diseases as Wernecke's encephalopathy, Korsakoff's psychosis, retrobulbar neuropathy, polyneuropathy and aspects of the pellagra syndrome - appears to be a dietary lack of the Vitamin B complex. Relative absence of the B vitamins produces virtually all of the symptoms of these diseases.

RATE OF ALCOHOLIC PSYCHOSES

Dr. Benjamin Malzberg (Research Foundation for Mental Hygiene), in a demographic study of alcoholic psychoses, finds that higher rates of alcoholic psychosis are associated with industrialization and urbanization. Occurrence of alcoholic psychosis is rare in people under 20 years of age. Dr. Malzberg confirmed the well-known fact that at all ages there are more men than women suffering from alcoholic psychoses.

A high percentage of patients with alcoholic psychoses who are admitted to hospitalization for the first time come from marginal economic circumstances. A comparison of occupations reveals an inverse relation between economic status and rate of first admissions. Those in the labor force who belonged to higher occupational categories had lower rates of first admission than did persons employed in such occupations as laborers and service workers. These data may well have to be interpreted in terms of the differing accessibility of treatment.

Dr. Malzberg found that, between the years 1940 and 1950, there was a significant decrease of alcoholic psychoses among Negro males hospitalized for the first time. The rate also decreased during this decade among white persons, but less than for Negroes. The significance of these decreases has yet to be determined. Dr. Malzberg also found that the foreign-born white population of the United States had a significantly lower rate of alcoholic psychoses than native-born white persons.

All of these statistical suggestions must be interpreted with caution. There are as yet no hard and fast data on the whole population of the United States. Dr. Malzberg's study referred to only a part of the population of hospitalized alcoholics, namely those with alcoholic psychoses. The findings are suggestive, but much more research needs to be done in the whole area of the epidemiology of alcoholism.

AGING

A large program of research in aging is currently under way at Duke University, where NIMH and NHI grants are making possible an integrated research effort to understand the nature of the aging processes. While this is a new program, some findings have already been reported.

AWARENESS OF AGE

One study of people's awareness of their own age conducted at Duke finds that the extent to which elderly people feel old is related to their physical status, the clarity of their thinking, their living arrangements, their vocational, avocational, and religious activity, and their actual age and feeling of physical health. They report that a person's awareness of his age depends more on psychological than physical factors.

INTELLIGENCE AND AGE

Another study growing out of the Duke program has established that the existing norms for the performance tests of people over 70 on the Wechsler Adult Intelligence Scale are possibly too high. The widespread use of the Wechsler Scale for intelligence testing gives this finding special practical significance. Studying 168 elderly people, the authors found constantly lower performance scores than the test standardization provided. NIMH's Laboratory of Psychology has been able to obtain the raw data for elderly people included in these standard tests. A careful examination of the adequacy of these norms for the elderly is under way.

AGE AND PHYSIOLOGY

Dr. William H. Knisely and his associates at Duke have reported their observations of blood vessel functioning of people over 60. They constructed an index based on circulatory factors, including the number of blood vessels per unit area, venous tortuosity, sacculations, arterial and venous agglutinated blood, and stasis. Studying 150 older people, they found that, while their index correlated positively with age, only four individuals meet all the criteria for normality. Their conclusion from this study is that either the norms were wrong or that the pathologies that were found were related to age.

BRAIN WAVE AND BLOOD PRESSURE

Other Duke University researchers studied the relationship of blood pressure to the pattern of electrical discharges of the brain as measured by the EEG. They find that aged patients with moderate elevations in blood pressure more frequently have normal EEG readings than do people with average or low blood pressures. These differences in blood pressure were most pronounced in cases where there were evidences of heart disease. These findings are related to previous studies showing a relationship between hospitalization for senile psychoses and decrease in cerebral blood flow. The authors suggest that this difference in blood pressure may be due to the extent to which the cerebral arteries are constricted.

SPEED AND FATIGUE

The Section on Aging at NIMH has continued its studies of the differences between aged and young organisms. Dr. James E. Birren and his associates report that the major single psychological factor which seems to be related to aging is speed of response. In studies of the swimming speed of rats of different ages and sexes, he was able to show a significant difference both in the swimming speed of old and young rats and demonstrated the increased susceptibility of older rats to fatigue. While practice reduced the fatigue effects in all groups, it had its greatest effects with all males, but did not change the maximum swimming speed. This suggests that the loss of speed with age may be physiological in origin, whereas fatigue may be a reflection of the living habits of the organism. It was also found that the older females, in general, were faster and fatigued less easily than did the males, which, while probably not causally connected, mirrors a general finding that older women tend to be more active than older men.

FLEXIBILITY

Dr. Jack Botwinick and his colleagues at NIMH have studied the relationship of age to the ability to shift from the performance of one task to another. Comparing elderly patients with young volunteers on a task which involved alternating from one kind of arithmetic problem to another, the investigators found that the aged subjects were significantly slower and the problems seemed harder to them. The finding is reminiscent of the folk saying that "it's hard to teach an old dog new tricks."

METABOLISM AND SOCIAL ADJUSTMENT

Bridging the gap between the behavioral studies and the physiological studies of aging is an investigation of healthy elderly men recently completed at NIMH, in which a relationship was found between the effectiveness of social behavior and measures of cerebral metabolism. Lower cerebral blood flow and cerebral oxygen consumption were found in persons who were functioning less well in their social environments, and who found leisure time and retirement oppressive.

PHYSICAL REFLECTIONS OF AGE

Paralleling studies of behavioral correlates of age are a variety of studies on concrete physiological reflections of the aging process. Drs. Joel Garbus and Eugene Weinbach at NIH have found several biochemical differences between tissues from old and young animals. A potentially important difference has been found in the level of total nucleotides and especially in the amounts of pyridine nucleotides. Older brain tissues contain less of these compounds which serve as electron carriers in cellular respiration.

Dr. Eugene Streicher, working at NIH, refuted an earlier theory by demonstrating that after the age of 12 months there is no significant increase in the calcium content of the rat's brain.

During this year a number of publications designed to stimulate research in the field of aging have been produced. A review of psychological research in aging is in press now; Dr. Birren has just completed a monograph on human aging, and is editing the first general handbook on the scientific understanding of aging.

PSYCHOSOMATICS

In addition to the continuing studies of the relationship of specific diseases to psychological stress, a number of studies have uncovered general and widespread physiological reactions to psychological events.

STRESS AND INFECTION

One such study reported by a group of NIMH grantees this year (Dr. A. F. Rasmussen et al., UCLA) subjected one group of mice to the stress of having to learn an avoidance reaction to electric shock and another to the stress of close and prolonged confinement. Within each group were subgroups subjected to the stress for 28 days, for 14 days, and for 1 day. In addition,

unstressed control animals were used with each group. At the end of these periods of stress, all the mice were inoculated with Herpes simplex virus, which is usually fatal to between 50 percent and 60 percent of normal mice within 14 days. Under both experimental conditions, mortality was significantly related to the amount of stress. Among the unstressed controls, mortality averaged 42 percent for the two control groups. Under both stress conditions, mortality increased with the amount of stress, reaching its maximum of 74 percent in the avoidance-learning group who were stressed for 28 days. From the study it appears that mice under prolonged stress do show increased susceptibility and less resistance to infection than do normals.

SUDDEN DEATH

In another experiment in the field of psychosomatic medicine, NIMH grantee Dr. Curt Richter of the Johns Hopkins University has attempted to clarify the phenomenon of sudden death frequently observed in primitive societies (voodoo death, death as a result of eating taboo food, death decreed by medicine men, etc.). Dr. Richter has succeeded in producing the same phenomenon in experimental animals, and infers from his study of the animals that it is related to overactivity of the parasympathetic nervous system.

After a series of chance observations of sudden death in rats, Dr. Richter attempted to set up laboratory conditions which would induce it experimentally. Caged wild rats were transferred to a black bag, were held in the experimenter's hand in an upright position, had their facial hair and whiskers clipped, and were put in a cylindrical swimming tank from which there was no hope of escape and in which they were forced to swim in a nearly upright position. Most of the wild animals died in a short period of time, while control domesticated rats survived in much greater numbers. All of these steps were studied as possible causes of sudden death and two appeared to be most important: restraint in the bag, and confinement in the swimming cylinder.

Electrocardiographic study of the rats, and control experiments using parasympathetic inhibitors and stimulants, and sympathicoadrenal and thyroid inhibition, all lead Dr. Richter to conclude that the sudden death response is one involving overactivity of the parasympathetic, rather than the sympathicoadrenal system. The situation and the reaction appears to be one of hopelessness rather than of fight or flight, and the animals seem to die because they literally have given up hope. This is supported by an experiment in which wild rats are restrained or immersed for very brief periods and then released. These animals learn that the situation is not hopeless, and they become aggressive and try to escape, rather than succumbing apathetically, as do rats shown no hope of escape.

Dr. Richter theorizes that the utter hopelessness of the voodoo death situation to the primitive man is comparable to the hopelessness of the rat's situation, and that the man dies of parasympathetic overactivity. Occurrences of sudden death of men at the sight of blood, upon hypodermic injection, or from sudden immersion in water sustain Dr. Richter's argument. The incidence of the sudden death response is much higher among men of more primitive societies, just as it is in undomesticated animals.

Dr. Richter's work, highly exploratory as it is, opens up a new area of research in the field of psychosomatic medicine, since it attempts to clarify the relationship between a particular psychological state and the physical response to it.

ASTHMA

Clinicians have suspected for years that emotional factors play a large role in asthma. Dr. Peter Knapp and his associates at Boston University have reported a high correlation between the severity of pulmonary disturbances and the severity of personality disturbances. In his study no patient was found with asthma of severe degree who did not also have major personality problems.

GROWTH DISORDERS

An interesting publication this year was produced by Drs. John L. Hampson and John Money of Johns Hopkins University, and is designed to assist the general physician in his work with children who are suffering from growth disorders. The authors have devised a five-category system for classifying growth disorders. For specific disorders in each category, the authors suggest general approaches in psychological therapy and case management. These psychological approaches often accompany somatic treatments where these will be effective, and call careful attention of the physician to the psychological problems with which these unfortunate patients must deal.

CANCER

A similar study was reported by Drs. Charles E. Orbach and Irving J. Bieber (Sloan-Kettering Institute). They describe the psychological reactions of patients who enter the hospital with the diagnosis of cancer. Many of these patients showed severe depressive and paranoid reactions which persisted after their cancer had been arrested and they had returned to their homes. These symptoms produced curtailment of the individual's effectiveness, and necessitated psychotherapy. The authors were able to identify the sources of these psychiatric reactions, and were able to facilitate psychologic rehabilitation.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

NEUROLOGICAL AND SENSORY DISORDERS

1958

Items of Interest on Research Studies Conducted and Supported by

the National Institute of Neurological Diseases and Blindness

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Neurological symptoms appear to stem from a multiplicity of causes, many of which are still unknown. Yet, it is encouraging to report concrete evidence of scientific research accomplishments, during 1958, directed to the cause, prevention, diagnosis, and treatment of neurological and sensory disorders.

The research developments discussed in the following pages represent some major achievements in laboratory and clinical investigations directed to nerve regeneration, the central nervous system, the brain and cerebral cortex, infant abnormalities, neuromuscular disorders, neuropharmacology, epilepsy control, cerebrovascular diseases, and disorders of vision; as well as progress in epidemiological studies on amyotrophic lateral sclerosis, encephalitis and related disorders.

The items reported here have appeared during the past year as published papers on studies conducted and supported by the National Institute of Neurological Diseases and Blindness.

NERVE REGENERATION

REGROWTH OF NERVE TISSUE POSSIBLE WITH MILLIPORE FILTER MATERIAL

Gaps up to one inch in the sciatic nerve of a cat can be bridged by functioning nerve tissue if shielded with a thin wall of filter material (Millipore). Reports of this method of nerve regeneration were given at the American Academy of Neurology meeting by Drs. James B. Campbell, C. Andrew Bassett, and Charles R. Nobeck, NINDB grantees at Columbia-Presbyterian Medical Center.

Prepared, January 1959

The process involves enclosing the severed nerve ends with the Millipore filter shield, a cellulose acetate plastic. The porous filter permits free exchange of nutrient material and blood plasma and prevents inflow of tissue cells which would block the path of nerve regeneration. The shield provides a kind of trellis supporting the new growth.

It has been possible to measure the amount of function restored in the cat's sciatic nerve. (This nerve activates muscles in the leg and foot.) One year after operation, function of the regenerated nerve approximates the normal when compared with the unoperated nerve.

Although nerve fibers will bridge one-fourth inch gaps in spinal cords of experimental animals, no return of nerve function in the cord has been noted after a year of observation. Myelin sheaths, the insulating material of some nerve fibers, appeared during the sixth month of regeneration. The investigators emphasized that to date only the anatomical union has been observed and that further time is needed to determine whether the spinal technique will permit return of function.

FUNCTION RESTORED TO PARALYZED DIAPHRAGM BY NERVE SUBSTITUTION

Functional restoration of the paralyzed diaphragm of animals has been accomplished by NINDB scientists in the Laboratory of Neuroanatomical Sciences through substituion of the nerve to the heart and viscera (vagus) for the nerve to the diaphragm (phrenic) by a process known as vagophrenic anastomosis. The procedure involves an arterial sleeve suturing technique using carotid arteries of other rats of the same strain.

A possible clinical application of such a procedure to patients with respiratory paralysis due to spinal cord disease, such as "bulbar" poliomyelitis, was suggested by the investigators. The report of their findings was presented at the meeting of the American Physiological Society in London, Ontario.

For purposes of this study, the vagus nerve was chosen as a substitute because it contains fibers having a discharge pattern similar to the phrenic nerve fibers. In applying this procedure to patients with respiratory paralysis, it was indicated that reinnervation of the diaphragm by the recurrent laryngeal nerve would appear to offer the greatest possibility of relief.

Motion picture records of the diaphragm of anesthetized rats provided a direct observation method for evaluating the diaphragmatic function.

SOME MOTOR FUNCTION RETAINED BY ANIMALS WITH PARTIALLY SEVERED SPINAL CORD

Cats with only one to ten percent of their lower thoracic spinal cord left intact still retained some motor function. The muscle tone and posture of these animals with partial transections were better compared to control animals which had total transections.

Scientists in the Laboratory of Neuroanatomical Sciences, NINDB, conducted their experiment on 13 cats after the animals' lower thoracic spinal cords had been partially severed. The report of these findings appeared in Neurology.

Three of the cats following operation had no motor function, but resembled those animals with total transections. A histologic study later revealed no intact nerve fibers at the site of operation and any neurons which may have been spared by the operation must have degenerated soon after.

Of the remaining ten cats, four had enough motor function to distinguish them from control animals, had good muscle tone and were not intensely spastic. This condition soon deteriorated, however, and a spastic paraplegic state followed. The other six retained varying degrees of motor function, sensory losses were complete, and no bladder function was present. These animals did not have hind limb spasticity which characterizes the usual paraplegic animal.

THE CENTRAL NERVOUS SYSTEM

NEW FINDING REPORTED ON IMPULSE TRANSMISSION IN SQUID NERVOUS SYSTEM

A phenomenon, described as the "vibration of the membrane current," has been observed and recorded, for the first time, in electrical experiments conducted by investigators in the NINDB Laboratory of Neurophysiology. The finding, reported in Science, suggests that the mechanism by which impulses are conducted along nerve pathways is very different from generally accepted theories. The experiments were done on the giant axon of the squid, which has served for most of the experiments upon which these theories have been based. The authors reported that they have observed the same phenomenon in experiments using toad nerve fibers.

The investigators used what is known as the voltage clamp technique in which the electrical potential (motive force) across the selected nerve-fiber membrane is measured and recorded. The electrical current (flow of ions) across the membrane, induced by the

application of a voltage clamp, is measured by micro-electrodes inserted into the nerve fiber.

When the membrane potential of the squid giant axon was raised by 10 to 15 millivolts above its resting level by clamping and was maintained for 10 milliseconds or longer, an inward surge of the membrane current was followed by a reversal of the direction of current flow. This surge-reversal process persisted often for six or more cycles leading to the conclusion that the current was oscillating or vibrating.

Sometime earlier, on the basis of other data, it was postulated that the nerve membrane tends to exhibit two relatively steady states. One is the resting potential and the other is a state of activity, which may be prolonged well beyond the normal action potential. The vibration effect observed represents current surges relating to the prolonged action potential state.

It is the hope of the investigators that these findings will contribute to the understanding of human nerve and nerve-muscle functions and of the manner in which the nervous system can become disordered.

BRAIN AND CEREBRAL CORTEX

CEREBELLAR DEGENERATION ASSOCIATED WITH CHRONIC ALCOHOLISM

A clinical study on the effect of alcohol on the nervous system indicates a close relationship between chronic alcoholism and cerebellar atrophy. All 35 patients involved in the study manifested a uniform cerebellar syndrome and revealed a history of prolonged and excessive drinking and of malnutrition.

A report of this study, by Dr. Raymond D. Adams, NINDB grantee, and associates at the Massachusetts General Hospital, Boston, was presented at the meeting of the American Neurological Association in Atlantic City.

Symptoms, dominated by leg incoordination and disturbance of gait, began at about 45 years of age. In the majority of the patients, the disease was reported to evolve rapidly. The maximum defects occurred within weeks or months with no change for years thereafter. Complete examination of the nervous system was made in four patients who died while under observation.

In general, the investigators pointed out that cerebellar symptoms progressed with continued excessive drinking while stabilization was attained with abstinence and improved nutrition. They concluded that the important etiologic factor may be malnutrition rather than direct effects of alcohol.

STUDY SUPPORTS CONCEPT OF A CENTRAL "CEREBRAL PACEMAKER" MECHANISM FOR SYNCHRONIZING BRAIN-WAVE ACTIVITY

The brain may have a subcortical mechanism, "the cerebral pacemaker," capable of regulating the synchronization of brain waves in the two cerebral hemispheres of humans. This mechanism may account for the high incidence of perfect synchrony of brain wave studies recorded from the two hemispheres simultaneously by high speed photographic methods. A lack of synchronization may indicate focal lesions in the brain and provide criteria for the selection of candidates for brain surgery.

A detailed analysis of 21,000 electroencephalograms, together with studies of synchrony in patients, was carried out by Drs. R. B. Aird and B. Garoutte, NINDB grantees at the University of California. A report of the study appeared in Neurology. Their findings tend to minimize the role of connecting fibers between the two hemispheres (corpus callosum) in maintaining synchrony, in favor of a mechanism located deeper in the brain, probably in the reticular substance of the upper brainstem or in the posterior hypothalamic region.

Generalized EEG changes or asynchronization may be produced by the influence of pathologic processes in the cortex. If the concept of a central pacemaker is correct, such generalized dysrhythmias should improve following surgery, as well as the patient's clinical status. The investigators conclude that focal asynchronism is as useful a criterion of focal abnormality as the out-of-phase activity of a focus obtained by linear or triangulation methods of recording.

FINDINGS SUPPORT NEURONAL BASIS FOR CEREBRAL SHOCK RESULTING FROM LOCALIZED INJURY IN THE CEREBRAL CORTEX

A localized injury in one area of the cerebral cortex may cause shock in another remote area of the cortex. Electrocorticogram recordings showed a depressive effect or a reduction of electrical activity due to an interruption in the fiber tract which connects the two areas. These findings support the neuronal basis for cerebral shock (diaschisis) as distinguished from the vascular basis.

The results of the study, obtained experimentally in anesthetized cats, were reported by Dr. Warren H. Kempinsky, a grantee at Washington University, St. Louis, in the A. M. A. Archives of Neurology and Psychiatry.

A number of factors related this depressive effect cerebral shock. According to Dr. Kempinsky, the depression was dependent principally upon a neuronal pathway, the corpus callosum, and occurred at a distance from the injury. A vascular cause was largely excluded by using two regions having separate blood supply. In addition, the fiber tract mainly responsible was identified, and the process was reversible.

Dr. Kempinsky suggests that the findings represent a particular case of a general process which also occurs with cerebral infarction and trauma in man.

CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION UNAFFECTED IN MENTAL AND NERVOUS DISORDERS

Cerebral blood flow and cerebral oxygen consumption may not necessarily be affected in individuals with deficient intellectual capacity, or individuals with central nervous system disturbances. Even those conditions attributed to disorders of cerebral circulation or metabolism may not have demonstrable changes in these values.

Dr. Joseph F. Fazekas, NINDB grantee at Georgetown University and cooperating investigators at District of Columbia General Hospital and the Children's Center, made a study of 7 mentally deficient individuals and 8 mongoloids in comparison with mentally normal individuals of approximately the same age. No significant differences were noted in these processes among the three groups tested. The report appeared in Neurology.

According to the study, it is possible, however, that small brain areas responsible for intellectual integration may be affected by seriously reduced cerebral blood flow or oxygen consumption rates without these changes being detected by the methods of measurement presently available.

RELATIONSHIP SHOWN BETWEEN LACK OF CEREBRAL BLOOD SUPPLY AND ANENCEPHALY

A NINDB grantee, Dr. F. Stephen Vogel, at the New York Hospital-Cornell Medical Center, has shown a definite relationship between abnormalities of the cerebral blood vessels and congenital absence of the brain (anencephaly)--the most common lethal congenital defect.

On the basis of autopsy of nine anencephalic fetuses and one two-headed fetus, with one normal and one deformed head, Dr. Vogel has concluded that vascular anomalies and anencephaly occur simultaneously. Previously, Dr. Vogel has shown that anencephaly in young embryos can be produced by disturbing the cerebral blood supply.

On the basis of the research findings and the recent human autopsy findings, Dr. Vogel stated that it is possible that the abnormal blood supply is an underlying factor in this lethal defect.

ISOLATED BRAIN, KEPT ALIVE BY AN ARTIFICIAL BLOOD SUPPLY, ALLOWS STUDY OF BRAIN FUNCTIONING

A NINDB grantee, Dr. Alexander Geiger, at the Neuropsychiatric Institute of the University of Illinois, has perfected a method of keeping alive a cat brain, which has been isolated from its blood supply. Artifical blood is introduced into the arteries to the brain and collected as it returns through veins of the brain.

The isolated brain continues to exhibit normal electrical activity and normal control over the muscles of the face. Further, the isolated brain continues to have normal blood pressure.

The addition of compounds, such as radioactive sugar, to the artificial blood, permits the study of the metabolic pathways concerned with the maintenance of energy supplies to the brain.

It is also possible to simultaneously produce changes in other organs of the body. Thus, Dr. Geiger is able to study not only normal brain functioning, but also brain functioning when other organs are impaired.

INFANT ABNORMALITIES

ABNORMALITIES IN OFFSPRING RELATED TO GERMAN MEASLES OCCURRING DURING PREGNANCY

Certain abnormalities in infants have been found to be specifically related to German measles (rubella) during early pregnancy. A comparative study by NINDB scientists, reported in Neurology, disclosed that the highest incidence of the three most important congenital anomalies--cataracts, deafness and congenital heart disease--occurred when rubella complicated pregnancy during the first five weeks.

495111 O-59-15

The study involved a group of 108 cases, analyzed from the literature, with evidence of brain damage from rubella acquired at various stages of pregnancy. These cases were correlated with a clinical diagnosis of an eight-year-old male whose abnormalities resulted from maternal rubella during the first four weeks of pregnancy. The patient experienced eleven pathological lesions out of a total of fifteen noted in the group studied.

The authors stressed the importance of the frequency with which the abnormalities in various organs were encountered relevant to different stages of gestation. There was a rapid decline of fetal susceptibility to the effects of rubella from the tenth week of gestation and no instances of fetal abnormality were encountered past twenty weeks.

It was pointed out that affected infants generally show various combinations of several of these conditions and it is uncommon to find only a single lesion. Another important fact noted was a distinct relationship between the severity of the rubella infection and the resulting damage even during the earliest stages of gestation.

EXCESS BLOOD AMMONIA IN INFANTS BORN OF RH NEGATIVE MOTHERS MAY BE A FACTOR IN BRAIN DAMAGE

Drs. Samuel P. Bessman and Martin K. Gorten, NINDB grantees in the Department of Pediatrics at the University of Maryland Medical School, are investigating the relationship between hyperbilirubinemia and high blood ammonia levels in children born of Rh negative mothers. Investigation of 16 children with hyperbilirubinemia severe enough to require exchange transfusion has revealed blood ammonia levels considerably higher than normal.

As a result of this study, these investigators postulate that elevated blood ammonia may cause a specific chemical lesion in the brain, thus rendering the brain susceptible to further damage by bilirubin. Once the brain is damaged by bilirubin, the lesion is irreversible, with the resulting effect being a cerebral palsied child.

Drs. Bessman and Gorten feel that treatment of these children with hyperbilirubinemia may include attempts to lower blood ammonia as well as exchange transfusion.

These investigators, and others, have previously shown that high blood ammonia levels are also found in cases with damaged or immature livers. Current research is also being directed towards investigation of liver function in normal newborns and in children born of Rh negative mothers.

NINDB STUDY EMPHASIZES IMPORTANCE OF DIAGNOSIS IN PROGRESSIVE CEREBRAL DEGENERATIONS IN CHILDHOOD

Cerebral regression in children does not necessarily indicate a latent birth injury, but may be due to an acquired progressively degenerative disease. Greater awareness of this factor in childhood regression may result in more cases being properly diagnosed, according to a recent study by Dr. Harold Stevens, Department of Neurology and Neurosurgery, George Washington University School of Medicine, and investigators in the Section on Developmental Neurology, NINDB.

In diagnosing the perplexing problem of the onset of progressive deterioration and neurologic defects in a previously healthy child the pediatrician or neurologist is often skeptical of a history that a child was healthy at birth and developed normally. Instead, he may draw the erroneous conclusion that a pre-existing birth injury manifested itself as the child grew older.

To aid in the precise diagnosis in this field, a reclassification of the syndromes is suggested in view of the advances in genetics, histopathology and biochemistry. In addition, a greater familiarity with the syndromes would also motivate more clinicians to pursue the study of an individual case to a definite etiological diagnosis.

The series of five cases reported revealed the symptoms in this group were not uniform. The investigators successfully used a series of tests in their diagnostic studies beginning with urinalysis, roentgenographic examination, electroencephalogram, pneumoencephalogram and finally, brain biopsy, now used only in selected cases.

Precise diagnosis would permit the accumulation of useful data on the diverse mechanisms of progressive cerebral degeneration in childhood and prove beneficial in treatment and prognosis. The latter is particularly important in family counseling in connection with the need for institutionalization and in providing parents with the genetics of the specific disease involved. The findings appeared in Neurology.

INFANTILE HEMIPLEGIA ASSOCIATED WITH SAGITTAL SINUS THROMBOSIS

A detailed report was presented on a case of infantile hemiplegia believed to have resulted from cerebral thrombosis of the sagittal sinus and its tributary veins in one hemisphere of the brain. To date, few such cases have been recorded.

Hemiplegia in early life is known to have occurred in apparently healthy individuals as a result of a sudden illness accompanied by severe convulsions. In some of these cases, thrombosis of the sagittal sinus may have occurred, the case now reported suggests.

In the case reported by investigators of the Surgical Neurology Branch, NINDB, hemiplegia followed a sudden illness with severe convulsions at the age of two and a half years. Paralysis persisted, mental development did not progress beyond imbecile level, and the patient experienced frequent epileptic attacks until his death at 28 years of age. A detailed account of the post-mortem examination appeared in the Journal of Neuropathology and Experimental Neurology.

It is generally known that occluded veins form new pathways and may become restored to normal in time. Therefore, the presence of organized thrombi (blood clots) in the sagittal sinus provides additional evidence that cerebral venous thrombosis had occurred in this case. They also discount the alternative of perinatal anoxic damage, since there was no evidence of Ammon's horn sclerosis in the right hemisphere nor patchy degeneration of the granular layer of the cerebellum.

An unexpected cerebellar finding in this case was an almost complete loss of Purkinje cells, which may have been due to anti-epileptic medication. This phenomenon can be produced experimentally in cats by administering dilantin in amounts comparable to maximal dosage used in man.

NEUROMUSCULAR DISORDERS

STUDY OF NEUROMUSCULAR DISORDERS CORRELATES AVAILABLE TECHNIQUES — General metabolic malfunction has been found to be the source of typical neuromuscular disorders in one-fifth of 166 patients studied.

This finding was one of the results of a survey of the effects of metabolic and endocrine abnormalities upon disease of the voluntary (striated) muscle. NINDB scientists conducted this study and their conclusions were presented at the meeting of the Association for Research in Nervous and Mental Diseases.

An attempt was made in this investigation to show where in the muscle structure these diseases may produce the effects that are observed clinically in neuromuscular disfunction. To accomplish this objective, clinical, biochemical and electrophysical techniques were used.

An example of the application of these techniques is given by the study of Familial Periodic Paralysis, one of the diseases considered in the survey. The investigation of this particular disease included a simultaneous study of the clinical progress of the disease; the level of certain important hormones; alteration in the ionic balance of sodium and potassium in the muscle fiber and the surrounding fluids; the microscopic appearance of the muscle during paralysis compared with normal activity; and the electrical activity of the muscle measured by fine intracellular electrodes. During the period of paralysis there was a striking accumulation of intracellular fluid without ionic imbalance and the muscle became electrically unexcitable. In association with an attack there was not an increased potassium excretion in the urine and in many cases administration of potassium proved beneficial.

The investigation revealed many unanswered questions concerning impaired function of the striated muscle, but with the techniques now available, the author concluded, more rapid progress in finding the answers should be possible.

PARKINSONISM

NEURONAL CHANGES AND TREMOR OBSERVED IN RESERPINIZED MONKEYS

Intramuscular injections of reserpine given daily to two monkeys for a period of 18 months produced almost continuous tremor. Postmortem microscopic examination revealed certain changes in the cell bodies and nuclei in some parts of the brain. However, no gross brain abnormalities were observed, according to investigators in the Laboratory of Neuroanatomical Sciences, NINDB.

In reporting the results of this long-term study at the American Academy of Neurology meeting, the investigators emphasized that these changes were characterized by enlarged nuclei, accumulation of unidentified material, and atrophy of nucleoli in the cerebral cortex, basal ganglia, and brain stem.

These changes were not confined to brain stem and basal ganglia as in human patients with paralysis agitans or Parkinsonism. The tremor resembles that of Parkinsonism, yet the neuronal changes induced by reserpine are more widespread than the neuropathological changes usually associated with Parkinsonism.

While it would appear that these changes reflect altered function of nucleus and nucleolus in certain neurons of the brain, the investigators state that it remains to be determined whether these changes represent a primary effect of reserpine on neurons, or a secondary effect through other systems. Preliminary observations in the cat confirm some of the studies which are being extended to other species.

MULTIPLE SCLEROSIS

EXPERIMENT REVEALS SUPPORTING STRUCTURE OF NERVOUS TISSUE MAY REACT TO STIMULI

Neuroglia cells, supporting structure of nervous tissue, may be capable of developing electric responses to direct stimuli as well as nerve cells. Scientists in the Laboratory of Neurophysiology, NINDB, reported in Science, an attempt made to record the electric responses from the neuroglia cells in the living cat.

The authors had previously examined nervous elements of the cat cerebrum and cerebellum in tissue culture under direct visual control. Electric responses then had been recorded in both nerve and neuroglia cells.

In this investigation, forceps were pushed diagonally into the cortex of the anesthetized cat to reduce movement of the cortex and to serve as one of the stimulating electrodes. A glass tube filled with Ringer agar gel was placed on the surface of the cortex between the legs of the forceps. The recording microelectrode was pushed into the region of the cortex where the density of the stimulating current appeared to be maximal.

The variations in electrical responses suggested to the investigators that the slow electric responses were similar to those of the neuroglia cells observed in the tissue culture.

The authors concluded that these experimental findings may raise many interesting problems in the field of brain physiology.

ELECTRON MICROSCOPIC STUDIES OF THE SYNAPSE CONCLUDE CONTROVERSY OVER NEURON DOCTRINE

New staining techniques and extensive use of the electron microscope have confirmed the neuron theory that the nervous system is constructed of independent units or neurons connected with one another by discontinuous contacts. The cleft between the nerve cells is shown to be about one-five millionth of an inch.

A NINDB neuroanatomist conducted his studies on tissue taken from the spinal cord and sections of the brain of the white rat. His findings were recently published in Experimental Cell Research.

The synaptic cleft was found to be extracellular space whose continuity can be traced among all the neighboring cellular structures. A thin layer of extracellular substance, presumably a fluid or thin gel, which has the properties of carrying transmitter substances and ions is found at the edges of the cleft. The cleft is open all around its circumference. However, there is no reason to expect that the shape of the cleft remains constant. During the actual transmission of a nerve impulse it may change in size and shape.

Since nerve impulse transmission has been one of the least known functions of the human body, the application of histochemical methods to electron microscopy may provide the means for solution of the problems of the synaptic processes.

AMYTROPHIC LATERAL SCLEROSIS

SKELETAL DEFORMITIES NOTED IN CASES OF AMYOTROPHIC LATERAL SCLEROSIS

The frequent occurrence of certain deformities of the hands and ears have been noted in association with amyotrophic lateral sclerosis. A possible biological-genetic explanation was presented by investigators of the Epidemiology Branch, NINDB, at the American Academy of Neurology meeting.

The authors have noted the frequent occurrences of a variety of deformities in two families in which extensive histories of amyotrophic lateral sclerosis were previously reported. In one family, a disfiguring anomaly of the hands was observed in four of nine siblings and abnormal development of the external ear and other abnormalities were observed in other members of this same family. In the second family, not fully investigated, hand deformities were observed in one member.

The incidence of similar deformities in the population with amyotrophic lateral sclerosis on Guam was reviewed and consideration was given to the possible biological-genetic explanation of these defects in motor neurone disease.

ENCEPHALITIS

COMPLICATIONS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALOMYELITIS MOST FREQUENT AND MOST SEVERE IN INFANTS

A five-year follow-up study of 750 cases of Western equine and St. Louis encephalomyelitis confirms earlier findings that the younger patient with a more serious and acute illness is more likely to have severe and permanent complications.

Included in the group studied by Dr. Knox H. Finley, NINDB grantee at Stanford University, were 270 children under the age of 10. Dr. Finley reported his findings at the American Neurological Association meeting.

Convulsions occurred during the acute phase in 95 percent of the infants up to two months old. Seizures recurred in more than half of those under one month and in nearly one-third of the 1-2 month group. However, about 40 percent of the children aged one to four had convulsions during the acute stage and only one-fifth of this group had recurrences.

Dr. Finley reported that the most common defects which may appear immediately or after a period of years are delayed or deficient speech, poor social adjustment, and intellectual impairment which may appear at school age.

NEUROLOGICAL SEQUELAE PREVALENT AMONG SURVIVORS OF 1947-48 ENCEPHALITIS EPIDEMIC ON GUAM

A high incidence of neurological or psychiatric abnormalities were noted in follow-up examinations of 43 survivors of three types of encephalitis present in the epidemic on Guam in 1947-48. Forty percent of Japanese B. encephalitis (JBE) survivors and 22 percent of mumps encephalitis (ME) survivors were found to have some neurological or psychiatric involvement. All four survivors of an unknown type of encephalitis had some neurological abnormality at the time of follow-up examination.

The findings were reported by an investigator of the Epidemiology Branch, NINDB, and Dr. Samuel J. L. Pieper, Jr., former epidemiologist, NINDB Guam Field Station at Agana. The report appeared in The American Journal of Tropical Medicine and Hygiene.

The examinations were conducted to determine the frequency and characteristics of sequelae and the prognostic value of several clinical features observed among the Guamanian hospitalized patients during the epidemic.

There were 46 known cases of encephalitis during the epidemic. Of the 43 survivors, thirty-two still live in Guam; records on six were available through military or civilian records and the remaining five are assumed normal in the absence of evidence to the contrary.

The sequelae noted in the survivors of JBE in this study were similar to those of an earlier study (Lewis et al). In decreasing frequency they were: impairment of the volunatry motor-nerve system

(pyramidal-system), mental retardation, impairment of cortical sensory association, brain-stem syndrome, cranial-nerve lesions, muscle atrophy, and convulsions.

Patients with ME suffered mild sequelae. Two patients examined had bilateral Babinski reflexes which suggests that ME may be one reason this pathological reflex is occasionally present in otherwise normal people.

Sequelae were much more common in the patients who were less than 10 years of age at the time of onset of JBE. Infants had more severe sequelae than older children.

The presence of convulsions and other signs of neuronal involvement during the illness correlated well with the later presence of sequelae of JBE. Other symptoms and signs such as elevated temperature, vomiting and spinal-fluid changes were not of particular prognostic significance.

The relatively low mortality rate of 7.4 percent and high sequelae rate of 40 percent (11 percent severe, 29 percent mild) observed in this report are in contrast to data from earlier Japanese studies on JBE which reported 33 percent or higher fatality and 3 to 14 percent sequelae. The authors concluded that the excellent medical care available to the population of Guam may have been responsible for the survival of many patients with severe brain damage who might have succumbed under other conditions.

EPILEPSY

REPORT EVALUATES ANTI-CONVULSANT THERAPY IN TREATMENT OF EPILEPSY

A NINDB grantee advocates the initial use of phenobarbitone, followed by hydantoin preparations or mysoline in major epileptic attacks. Dr. H. Houston Merritt, Director of graduate training in Clinical Neurology, College of Physicians and Surgeons, New York, states that trimethadione is most effective in lesser attacks. He bases his conclusions on a study of 319 patients with epilepsy treated with various anticonvulsants. In the group under study, seizures were controlled in 154 patients (48 percent), improved in 118 (37 percent). No improvement was noted in 47 patients (15 percent).

Dr. Merritt briefly described the history of anticonvulsants, and discussed the more recent drugs. The first effective one was bromide, used as early as 1859. Phenobarbitone was first used in 1912 and hydantoin was introduced by Drs. Putnam and Merritt in 1937.

Phenacetylcarbamide is useful in treating temporal lobe epilepsy although it produces toxic side effects. Milontin and Diamox are both useful in patients with petit mal. The three principles which guided his treatment of epilepsy in the study were the removal of causative and precipitating factors where possible, the establishment of regular physical and mental habits, and the use of appropriate drugs. Most cases could be controlled with one or two remedies. However, in rare cases as many as four were given.

NON-METHYLATED COMPOUNDS FOUND AS EFFECTIVE IN EPILEPSY TREATMENT AS COMMERCIAL METHYLATED DRUGS

A treatment for epilepsy which is less expensive, yet, equally effective as those now in use was reported by NINDB grantees. A study of antiepileptic drugs has revealed that it is doubtful whether commercially available methylated drugs are of more value therapeutically than non-methylated compounds. Methylated compounds are eventually demethylated in the body and there is no convincing evidence that they permit a lower incidence of toxic reactions than the non-methylated drugs.

Drs. Thomas C. Butler and William J. Waddell of the University of North Carolina state that although demethylation proceeds at a slow rate in the body, the product is eliminated at an even slower rate. Therefore, according to the investigators, administration of the methylated drug in a chronic schedule results in a more extensive accumulation of demethylated product than of the original drug.

An example of a demethylated product known to be a clinically effective antiepileptic agent is phenobarbital, the demethylated product of mephobarbital. A report on the measurement of the concentrations in the plasma of human subjects of this compound and four others now available for use with epilepsy patients appeared in Neurology.

ESTROGEN INCREASE IN THE BODY AT MENARCHE MAY CAUSE EPILEPTIC SEIZURES; PROGESTERONE MAY ACT AS PREVENTIVE

Estrogen, which increases in the body at the premenstrual period, may be directly responsible for the aggravation of seizures. Progesterone, given premenstrually, may prevent such attacks. These findings have been demonstrated in experiments with both humans and animals by Dr. Frank Morrell, Dr. John Logothetis, NINDB grantees, and associates at the University of Minnesota Medical School. The report was presented at the American Academy of Neurology meeting.

The results of this study suggest that if catamenial (menstrual) seizures are precipitated by an increase in estrogenic activity at the

premenstrual period, it is possible that hormones having antagonistic properties to estrogens, such as testosterone and progesterone, may help prevent the attacks.

Following injections of Premarin (estrogen substance), eleven of sixteen patients demonstrated abnormalities ranging from EEG activation during and immediately after injection to actual seizures occurring 15 minutes to 12-24 hours after injection. The application of Premarin directly over a chronic epileptic lesion in rabbits resulted in immediate increase of the convulsive electrical discharge with tendency to spread and produce a clinical seizure.

An additional practical value may be the usage of Premarin injections for diagnostic activating electroencephalographic studies.

VISUAL FIELD RELATIVELY UNIMPAIRED BY TEMPORAL LOBECTOMY IN EPILEPSY CASES

A study involving 41 patients in whom the temporal lobe had been removed for epilepsy relief shows visual acuity to be an area essentially unchanged by lesions in the temporal lobe. The study suggests that the visual field defects observed in these cases are the result of a lesion solely of the optic radiation (fibres from the lower visual centers which pass backward to the occipital lobe).

An extensive report of these findings by investigators of the Surgical Neurology Branch, NINDB, appeared in BRAIN.

Regarding the functional nature of the visual fibres affected by lesions of the temporal lobe in man, the investigators conclude that the tip of the temporal horn may be surgically excised without producing a detectable change in the field of vision.

Throughout the greater part of the study, considerable use was made of the Gunkel self-recording tangent screen, a device which was developed by a staff member of the Ophthalmology Branch, NINDB, for charting the visual field.

LEFT TEMPORAL LESIONS PREDOMINANT IN EPILEPTIC PATIENTS WITH COMMUNICATION DEFICITS

Patients with an epileptiform lesion in the area of the left temporal lobe were found to have difficulty in interpersonal communication, members of the NINDB Surgical Neurology staff reported at the meeting of the American Speech and Hearing Association held at New York.

The purpose of the NINDB study was to determine whether there exists a demonstrable communication deficit in some epileptic patients, to discover whether such a deficit might be related to a lesion in some specific area of the brain and to decide whether a lesion in such an area, though related to communication, might differ from a true aphasia.

Seventy-three patients with cerebral seizures who had responded unsatisfactorily to medical therapy and were awaiting surgery were chosen for the study.

Before the medical examination of each of the 73 patients was completed, judgment was made as to the adequacy of their interpersonal communication. Thirty-two of the patients were found to be 'poor' communicators, four of which had aphasic symptoms between seizures, and the remainder were considered 'adequate'.

When a comparison was made later between the judgments of the quality of communication and the site of the eventual operation, it was seen that 93 percent of those patients who had removals of the left temporal lobe had been considered 'poor' communicators. Only 13 percent of the patients who had removals of other areas of the brain were so judged.

RELATIONSHIP REPORTED BETWEEN ADRENAL ACTIVITY AND CONVULSIONS

It has been found that epileptic patients excrete abnormally small amounts of adrenal hormones in their urine. Other experiments indicate the importance of these hormones in influencing the excitability of the brain.

The findings of Dr. Patrick F. Bray and other doctors at the University of Utah, College of Medicine, were reported at the meeting of the American Academy of Neurology. Further research is necessary, however, to determine whether the adrenal abnormality causes the seizure or whether it is a compensatory activity of the body to counteract the epilepsy.

These findings are of interest to a large segment of the population since one out of every 200 persons suffers from epilepsy and 5 percent of all people have had at least one seizure.

NEUROPHARMACOLOGY

CHEMICAL COMPOUNDS (OXIMES) USEFUL ANTIDOTES AGAINST NERVE GASES

A number of chemical compounds have been found effective in overcoming the poisonous effects of certain insecticides and nerve gases, according to NINDB grantees at JohnsHopkins Hospital. Drs. David Grob and R. J. Johns reported their findings at the American Academy of Neurology meeting.

Certain insecticides such as parathion, and chemical warfare agents such as sarin (nerve gas), produce paralysis by blocking the passage of the nerve impulse message to the muscle. Among the compounds (oximes) which rapidly and effectively counteract the poisons are pyridine-2-aldoxime(2-PAM) and diacetyl monoxime (DAM).

These compounds may provide protection in time of war and to farmers and agricultural workers against widely used insecticide sprays.

STIMULANT FOUND TO REVERSE SEDATIVE ACTION OF PROMAZINE; MAY SERVE AS ANTIDOTE TO DEPRESSION IN TREATMENT OF PSYCHOMOTOR DISORDERS

NINDB grantees reported the results of a study of 21 patients to whom a tranquilizer (promazine) and a stimulant (methylphenidate hydrochloride) had been given. The grantees included Drs. W. R. Ehrmentraut, J. G. Shea, H. E. Ticktin, and J. F. Fazekas from the George Washington and Georgetown University Medical Divisions and the District of Columbia General Hospital.

The drugs, given separately and in combination, were found to have no significant effect either on cerebral blood flow or oxygen consumption. Methylphenidate, however, was found to be effective in reversing the depressing action of promazine. Such a depression may lead to confinement in a mental hospital or to suicide.

It was pointed out that the new tranquilizer, promazine, appears to offer considerable promise in the control of physically overactive patients. Furthermore, the patient's response to methylphenidate, after sedation with promazine, generally consisted of increased alertness, ease of speech, or improved articulation. These results suggest the usefulness of methylphenidate as an antidote to depression caused by the tranquilizers of the phenothiazine group.

SODIUM BICARBONATE FOUND TO BE EFFECTIVE ANTIDOTE FOR OVER-DOSAGE OF PHENOBARBITAL

Phenobarbital poisoning can be treated effectively through intravenous infusion of a concentrated solution of sodium bicarbonate.

Drs. William J. Waddell and Thomas C. Butler, NINDB grantees at the University of North Carolina School of Medicine, in a report to the Journal of Clinical Investigation, indicated the procedure involves minimal risk and requires no special equipment.

The investigators have shown that sodium bicarbonate can shorten phenobarbital coma by rendering the body more alkaline. In deep coma the blood pH (hydrogen ion concentration) is lowered allowing more phenobarbital to enter the brain and extend the depression. Raising the blood pH with sodium bicarbonate lightens the depression and allows some of the phenobarbital to pass from the brain.

Sodium bicarbonate was also found to increase the rate of elimination of phenobarbital, normally the most slowly eliminated of the commonly used barbiturates. This alkalinizing treatment, however, was found to be less effective with other barbiturates.

HEADACHE

ACETYLCHOLINE FOUND IN CEREBROSPINAL FLUID OF PATIENTS WITH MIGRAINE AND VASCULAR HEADACHES

A clinical study supports a basic theory that acetylcholine may be the chemical agent directly responsible for arterial dilation in migraine and vascular headaches.

Dr. E. Charles Kunkle, NINDB grantee at Duke University, presented his findings at the American Neurological Association meeting in Atlantic City.

Dr. Kunkle collected samples of cerebrospinal fluid from 22 patients with varieties of migraine headache, during and between attacks, and from a control group of 15 patients with non-vascular headaches and 37 patients with various neurological disorders.

Among the 22 patients tapped during vascular headaches, an acetylcholine-like effect was demonstrated in 5, all from a subgroup of 9 with headaches apparently originating in intracranial arteries. In amount it was equivalent to 0.004-0.06 micrograms of acetylcholine per milliliter of cerebrospinal fluid. No acetylcholine was detected in samples from the control group.

CEREBROVASCULAR DISEASES

CEREBRAL ANEURYSM MORTALITY DRASTICALLY REDUCED BY NEW SURGICAL TECHNIQUE

Lowering the body temperature to 27-29° C. and temporarily closing the main branches of the cerebral arteries (circle of Willis) is a relatively new technique applied in surgery of ruptured aneurysms. Fifteen successive cases were described by Dr. J. Lawrence Pool, NINDB grantee at Columbia University. This technique can reduce mortality in such cases from 50 percent to 17 percent.

While the body temperature is lowered, it is safe to close the circle of Willis for as long as 15 to 20 minutes. Some degree of circulation remains in the middle and posterior cerebral arteries to provide some protection to the cortex which is temporarily deprived of anterior cerebral artery flow. After dissection of the aneurysm and placement of permanent metal clips, the temporary clips are then removed and the wound is closed.

Dr. Pool pointed out that massive intraventricular bleeding usually caused death within 48 hours or so, of at least 25 percent of all patients with subarachnoid hemorrhage due to ruptured intracranial aneurysm. With no surgical treatment, another 25 percent would die within three weeks of onset and an additional 20 percent may be expected to die or be permanently crippled by recurrent hemorrhage. This technique, presently used by other doctors in the country, aids early permanent obliteration of intracranial aneurysms that have recently bled.

EARLY SURGERY MAY PREVENT STROKES CAUSED BY NECK AND CHEST BLOCKAGE

Immediate recognition and early removal of partial clots in arteries leading to the head may prevent brain damage by stroke, reported an NINDB grantee at the American Academy of Neurology meeting.

Dr. William F. Fields of Baylor University College of Medicine, Houston, Texas, stressed the importance of early diagnosis of partial blocks in arteries of the neck and chest before a stroke occurs. Once the occlusion spreads upward beyond the neck, he said, surgical correction is no longer possible.

Reporting on research done in collaboration with Drs. Michael E. DeBakey and E. Stanley Crawford of Baylor, Dr. Fields cited twelve of thirteen recent operations as successful. In these cases, the partial clots were removed by performing surgical grafts to bypass localized occlusions in the arteries leading to the head.

Important early symptoms of occlusion are a rhythmic swishing sound in the neck (audible with a stethoscope) in older people, and a transient loss or diminution of vision in one eye. Dr. Fields pointed out that accurate diagnosis is often impossible without cerebral angiography.

ANTICOAGULANT THERAPY FOUND BENEFICIAL IN EARLY STAGES OF CEREBRAL THROMBOSIS

The use of anticoagulant drugs may be a promising therapy in the earlier stages of cerebral thrombosis. In a clinical study involving 58 patients, the drugs appeared to abolish transient ischemic attacks (blood deficiency due to vessel contraction) and may prevent or postpone the onset of a threatening stroke.

A detailed report by Dr. C. Miller Fisher, containing illustrative case histories, appeared in Neurology. Dr. Fisher is a grantee of the NINDB at the Massachusetts General Hospital.

The patients, observed for periods both on and off anticoagulants, had symptoms and signs of various stages of cerebral thrombosis due to atherosclerosis. A group of 37 comparable cases without anticoagulant therapy was observed to follow a far less satisfactory course than the treated patients.

According to Dr. Fisher, the anticoagulants seem more beneficial than any other therapy used at present and they are relatively safe.

DISORDERS OF VISION

IRRADIATION OF LENS TISSUES MAY PRODUCE CATARACTS

Lens tissue changes in the eye are evident immediately following medium doses of irradiation and usually lead to cataract formation. At the same time, other ocular tissues appear to escape signs of permanent damage.

A NINDB ophthalmologist reported his findings at the sessions of the American Academy of Ophthalmology and Otolaryngology.

Cell division (mitosis) in the lens epithelium is markedly decreased ten minutes after exposure of the eye to a dose of about 1500 units (rad) x-rays. Mitotic inhibition is complete about 50 minutes after irradiation. The duration of suppression of this process is dependent upon dosage.

Of even greater cytopathologic significance than the effect on mitosis are early signs of injury to the cell nuclei. The number of cells injured increases with the dose up to 1000 rad x-rays, which appears to be the saturation level. Some damaged cells are completely destroyed, while other nuclei apparently suffer only small losses of chromatin substance. These cells may recover, survive, divide and differentiate. It is possible, however, that the daughter cells do not develop healthy fibers, which in turn could produce cataractous changes after long periods of time.

The effect of x-irradiation on the epithelium as seen under the light microscope was compared with that seen with the electron microscope. Early cytoplasmic changes are evident only with the second technique.

Two experimental approaches have been used to demonstrate directly the difference of vulnerability of various regions of the lens epithelium to ionizing radiations. In one approach, lead shields with different cutouts were fastened to the eye before it was placed in the x-ray beam. In the other, high energy particles were directed to selected parts of the lens. These particles exhibit less scattering than x-rays and allow aiming of the beam at circumscribed sites. Cytological studies have been carried out only with the second technique. In this method the germinative zone is outside the beam and reduction of mitosis does not exceed 50 percent of that of the control eye and recovery is complete on the fourth day. Irradiation of the germinative zone through the first method results in complete inhibition of mitosis for six days and takes 14 days before normal mitosis is established.

The difference of radiosensitivity of various parts of the lens epithelium is of greatest importance when ionizing radiations of limited penetration are used. Some comprehension of the dose dependency of radiation injury and of the relative sensitivity of zones of the lens epithelium is essential for treatment of eye tissue with this physical agent. Serious complication of a therapeutically produced radiation cataract is unavoidable unless these two factors are taken into consideration.

CHANGES IN RABBIT LENSES FOUND TO BE CHARACTERISTIC OF THE AGING PROCESS: SHEDS LIGHT ON SENILE CATARACT

A NINDB grantee reported on a comparative age study of rabbit lenses. This study was in line with recommendations strongly urged at an NINDB-sponsored Symposium on Aging that comparative studies be made on animals of known ages.

The findings of Dr. Harry Green of the Wills Eye Hospital, University of Pennsylvania, constitute a major step in the search

for the responsible factor in the development of senile cataract, one of the most common types of ocular ailments in older persons. It is the generally accepted concept that the transparency of the lens is dependent upon the active metabolism (use of food substances) of the tissue. It was pointed out, however, that practically no information is available on normal human lens metabolism.

Extracts of lenses from 1-month old rabbits were compared with those of 3-year old rabbits as to their ability to produce lactic acid from glucose and other specific substances. Lactic acid is one of the end-products in the metabolism of the lens.

On the basis of the whole lens, the older lenses have a greater content of active enzymes, thus, a greater capacity to produce lactic acid. However, the younger lenses showed the greater metabolic efficiency when calculated on the basis of activity per 100 mg. wet weight or per milligram of protein. These chemical changes appear to follow what is known about the aging process in the normal, transparent lens.

SUCCESSFUL SURGICAL TREATMENT OF IRIS TUMORS REPORTED

Small tumors of the iris of the eye, in most instances, can be completely removed by surgery with excellent recovery. In cases where there has been recurrence or a complicating factor exists and the eye must be removed for the patient's welfare, recovery is still relatively good.

Tumors located at the outer surface of the iris should be excised early to prevent growth into the ciliary body. Those tumors that are well defined, particularly in the pupillary and midzonal portions of the iris, need not be treated at all unless there is evidence of growth or local disturbance such as cataract. Iris tumors are unusual and statistical data difficult to compile, but with the gradual accumulation of this material and sufficient follow-up periods, there is a better understanding of the natural behavior of these tumors.

Dr. Benjamin Rones, Director of the Ophthalmic Pathology Laboratory, Washington Hospital Center and Dr. Lorenz E. Zimmerman, Chief, Section on Ophthalmic Pathology, Armed Forces Institute of Pathology, NINDB grantees, have evaluated the results of iridectomy in a series of 125 cases taken from the files of the Armed Forces Institute of Pathology. Their criteria for this study was based on cases in which iridectomy had been the initial

surgical procedure for treatment of a localized tumor, sections of the tumor were available for review, and the tumors were of the broad category of melanotic or potentially pigmented neoplasms.

The study revealed that patients with melanotic iris tumors were younger than those treated for other tumors of the eye. Two-thirds of iris tumors, sufficiently small and localized enough to be removed by iridectomy, were benign. These tumors may be infiltrative and locally destructive, but they probably never metastasize. These remaining tumors contained malignant cells. However, only three of 38 such tumors in the groups are believed to have metastasized. According to the investigators, the life expectancy of the patient with iris tumor is rarely affected if conservative treatment is followed.

Drs. Rones and Zimmerman's findings and case histories are published in the American Medical Association Archives of Ophthalmology.

STUDIES REVEAL NEW INFORMATION ON LIGHT TRANSMISSION FROM THE EYE TO THE BRAIN

An analysis of the electrical activity within the cells of the eye of invertebrates has revealed that nerve impulses are generated as a consequence of permeability change of the membrane.

These findings were presented by a staff member of the Ophthalmology Branch, NINDB, in the annual Bishop Lecture of the Washington University Medical Center in St. Louis, Missouri.

In the eye, the energy of light causes the membranous coat of the receiving part of the nerve cell (the dendrite) to lose some of its polarization or resting electrical charge. Subsequently, the nerve impulse to the brain is generated from a different part of the cell, the central axon. The reception of light and the generation of the nerve impulse are linked with a change in permeability of the cell membrane to ions such as sodium and potassium.

Several of the mechanisms involved in generation of impulses in receptors appear to be similar to those responsible for impulse generation in the central nervous system. It is hoped that further study of these receptors will reveal mechanisms which are important for the function of all nerve cells.

HYALINE CORPUSCLES IN THE HUMAN OPTIC NERVE MAY BE EVIDENCE OF NERVE-FIBER DEGENERATION

NINDB grantees make use of a new silver staining technique to study the formation of hyaline bodies in a patient with optic-nerve atrophy resulting from a brain tumor.

Drs. J. Reimer Wolter and Leopold Liss, Ophthalmology Department of the University of Michigan, observed in this case that many of the surviving nerve fibers did not run up to the brain, as they normally do, but were interrupted at different points along the pathway of the optic nerve. At the proximal end of each interrupted fiber, there first appeared a swelling composed of nerve-fiber substance and free lipoids that later underwent hyalinization. Concomitently, there was a retrograde degeneration of the distal portion of the nerve fiber, leaving free the hyalinized corpuscle.

Previous work by Drs.Wolter and Liss has shown that similar hyaline bodies may result from a process of degeneration of astrocytes within the optic nerve tract, as well as the above mentioned process of degeneration of nerve fibers. These studies add further information on the nature and mechanism of formation of hyaline corpuscles, a common finding not only in the nerve fiber but also in the retina of many older persons.

APPLICATION OF NEW TECHNIQUES

HYPOTHERMIA FOUND TO REDUCE CEREBRAL EDEMA

Cerebral edema, a major neurosurgical problem, has been materially reduced by hypothermia.

Following the use of this technique in artificially produced brain lesions in animals, edema was reduced during the first 48 hours.

An investigator of the Surgical Neurology Branch, NINDB, presented his findings at the Clinical Congress of the American College of Surgeons at Chicago. Increased use of hypothermia in neurosurgery prompted a study of its effect on the development and activity of edema in brain lesions.

To assess the effects of hypothermia, the cats used in the experiment were divided into three groups. The brain lesions artificially produced in these animals were accomplished by the application of a -55 degree centigrade plate to the cortex. Hypothermia was not used in the first group serving as a control. The second group was operated on after hypothermia was induced to correspond with usual neurosurgical practices. In the third group the lesion was produced six hours before induction of hypothermia simulating clinically its potential therapeutic use following brain injury.

The permeability of the blood-brain-barrier and reaction of the tissue elements of the hypothermic animals were less at 24 hours after operation than in the control animals. The hypothermic effect at 48 hours was not as great as at 24 hours, but there was still less reaction than appeared in the control animals at 24 hours. Thereafter, the characteristics of edema subsided more quickly than in normal controls.

Further studies on the effects of hypothermia are still in progress at NINDB.

NERVE MEMBRANES ANALYZED BY COMPUTERS

Analog computer concepts and components are now being used to control active nerve membranes and to measure accurately and rapidly their nonlinear conductances, capacitance, series resistance, and other properties. Rapid recording of this data is imperative since biological preparations last only a few hours and deteriorate rapidly.

New uses of computer concepts were presented by a NINDB biophysicist at the Conference on Electrical Techniques in Medicine and Biology at Minneapolis.

Chopper stabilized operational amplifiers are used in the Laboratory of Biophysics to control the voltage and current applied to the membrane. This allows the electrical properties of the membrane to be determined under controlled conditions. Chopper stabilization also is applied to a potentiometric-type amplifier which provides very high input resistance and capacitance neutralization. The amplifiers and other components connected to the membrane through a special purpose program board form a real time computer.

HIGHLIGHTS OF

CLINICAL CENTER

RESEARCH

1958

Items of Interest on Research Studies Conducted by the Clinical

Center

Two axioms of modern medical research are:

(a) Laboratory study of basic life processes is the foundation of all effective attacks on urgent current problems of human health.

(b) Promising new medical developments arising from basic research must be thoroughly evaluated by clinical studies under hospital conditions.

The enormous expansion of manpower, facilities, and supporting funds for medical research during the past decade has been properly concentrated mainly on the study of basic phenomena. A constantly swelling volume of promising new concepts, methods, devices, and compounds is beginning to emerge from this creative process. Increasingly hospitals throughout the country are being involved in the next step--the clinical evaluation which must precede routine universal utilization of new knowledge.

The Clinical Center of the National Institutes of Health has in the $5\frac{1}{2}$ years of its existence developed many new principles and techniques of value to other hospitals which are engaging in large scale clinical research.

Typical of the contributions by the NIH Center are those originating in its Department of Clinical Pathology. Of considerable significance is the fact that many of these contributions will help provide more efficient and effective service by all hospitals as well as by those involved in organized research.

Prepared, January 1959

The Clinical Pathology Department of the Clinical Center provides laboratory consultation and services of research precision in the areas of hematology, clinical chemistry, microbiology, and special diagnostic procedures. Experience has shown clearly that routine and special tests on research patients are required much more frequently, are more varied, and must be more accurate than those needed for patient care in a general hospital.

A recently invented electronic counting instrument has been modified by the Department and evaluated for counting red cells, leukocytes, and tumor cells. With this instrument a count of high accuracy is made in 15 seconds as compared with 5 minutes when done by the best trained technologist using customary microscopic methods. Automatic pipetting and flame photometry for both sodium and potassium on a single serum dilution of a specimen further speeds up these procedures.

Advanced techniques from the field of research and industrial chemistry have also been taken over by the Clinical Pathology Department. An example is the Cotlove chloride amperometer, developed at the National Heart Institute, which enables chloride determinations to be made of sweat, serum, blood, and urine quickly and accurately (1 minute per determination). One very important advantage in its use on hospitalized patients is that for blood chloride determination it requires only one-tenth the amount of blood demanded by older methods.

Another apparatus adopted by the department is the autoanalyzer, which mechanically determines blood glucose, nitrogen, and phosphate levels, automatically calculating and recording the results. This device is accurate, fast, and occupies very little space. Three such instruments and two part-time technologist operators now perform chemical determinations that formerly demanded the full time of 6 technologists.

In addition to providing services crucially important to the research patient's contribution and welfare, this department is aiding the entire field of medical diagnosis through its program of adapting advanced research laboratory techniques and developing standards of precision in the field of clinical pathology.

HIGHLIGHTS OF PROGRESS

IN BIOLOGICS CONTROL

1958

Items of Interest on Research Studies Conducted by the

Division of Biologics Standards

The Division of Biologics Standards is responsible for the administration of those provisions of the Public Health Service Act pertaining to the control of biological products that enter into interstate or foreign commerce. This regulatory function is discharged through a licensing procedure whereby both establishment licenses and product licenses are issued, following a determination by the Division that the prescribed standards of safety, purity, and potency have been met. At the end of the calendar year, 169 establishment licenses and 1,209 product licenses were in effect, the latter covering 272 specific products. In the first eleven months of 1958, tests of 3,183 individual lots of various biological products were made, ranging from relatively simple sterility tests to potency determinations involving both animal and tissue culture tests. Necessary to an effective control program are the supportive research activities of the Division essential to the eventual licensing of new immunizing agents as they are developed and to the continuous improvement of those already established.

CHICK TEST RELIABLE FOR ASSAY OF POLIO VACCINE POTENCY

The chick potency test for poliomyelitis vaccine developed by DBS scientists has been found to ensure reliable measures of potency during a year of parallel testing with the presently required monkey potency test by DBS and vaccine manufacturers. Because the monkey potency test, although adequate, is too complex and costly for large-scale investigations necessary for definitive studies of the vaccine's potency and stability, a study was made in 1957 to determine whether any animal other than the monkey could be successfully used for assay of all three virus strains of the vaccine. The baby chick was found to be the most promising test animal for all types of potency tests required.

Prepared, January 1959

For the past year, with the advice of the Technical Committee on Poliomyelitis Vaccine, vaccine manufacturers have used the chick potency test concurrently with the monkey potency test on a trial basis. The data on parallel testing clearly indicates that the chick test is a less complex assay procedure, ensuring reliable measures of potency for all three vaccine strains. The results have been found to be similar to those of the monkey test, with approximately the same degree of variation in individual tests. The use of the chick obviates the inherent disadvantages of the monkey, such as difficulty of procurement, attrition, lack of homogeneity, costly feeding, housing, and care, thus providing the ability to increase the precision of a test by employing large numbers of animals. Recommendations to adopt the chick test as the official potency procedure will be submitted to the Technical Committee at an early date.

TEST-TUBE POTENCY TEST DEVELOPED FOR POLIO VACCINE

An in vitro potency test for poliomyelitis vaccine based on the observation that antibodies will combine with either virus or vaccine has been developed in DBS laboratories. This test is a valuable addition to the techniques available for testing the vaccine for antigenicity in that, since it is a test-tube technique, it is simple, economical, and reproducible from one test to the next--advantages which the animal potency test does not have.

The test is based on work of Dulbecco, Krech, and McLean, but modified to take into account Gard's recent findings on immuno-inactivation. Prolonged incubation is essential to complete the neutralization of the poliovirus by its antibody, or similarly, in the present instance, to complete the binding of the antibody by the vaccine. The test is carried out as follows: A fixed amount of antibody is added to varying dilutions of vaccine. Vaccine-serum mixtures are incubated at 37° C for 6 hours, then refrigerated overnight at 4° C. A known number of infectious virus particles are added, incubated for an additional 6 hours at 37° C, refrigerated overnight again, and the residual infectious virus assayed by the plaque method on monkey monolayer cultures.

If the vaccine is relatively potent, the majority of the antibodies will have been bound by the vaccine prior to the addition of the virus, and little or no antibodies will be left to combine with the virus. Consequently, a large amount of residual infectious virus indicates a vaccine of high potency while a small amount indicates a vaccine of poor potency. For purposes of standardization, a reference vaccine is always tested in the same way as a basis of comparison.

Since the antibody-combining test does not directly measure the ability of the vaccine to stimulate antibody production in living animals, such as monkeys and chicks, it is necessary to establish that the test actually measures this ability. This is done by a series of tests in which vaccines are assayed for potency in animals and also in comparative antibody-combining tests.

IN VITRO METHOD DEVELOPED TO TELL VIRULENT FROM ATTENUATED POLIO VIRUS

A new method of distinguishing virulent from attenuated polioviruses without using living monkeys is being studied by DBS scientists. The growth patterns of the three strains of poliovirus (Mahoney, MEF, and Saukett) used in the preparation of poliomyelitis vaccine, and those of three attenuated strains (one of each serotype) have been determined in primary monkey kidney tissue culture and in a continuous stable cell line derived from monkey kidney.

It has been found that all six virus strains yield approximately the same titers in primary monkey kidney and in continuous stable cell line (HeLa) cultures in fluid medium, but in the HeLa culture the attenuated strains and the Saukett strain yield titers as low as 0.01 percent of the values obtained with the highly virulent Mahoney and MEF strains.

When assayed by the plaque method (enumeration of virus colonies) on usual monkey kidney monolayers, all six strains yield approximately the same titers. On monolayer cultures of the continuous cell line, however, the attenuated strains show fewer virus colonies than do the virulent strains, and the size of the plaques or colonies become a distinguishing genetic marker of attenuation. While the highly virulent Mahoney and MEF strains produce plaques of 3 to 5 mm. in diameter, the other four virus strains yield plaques less than 1 mm. in diameter.

The Division expects to use this method of identification to determine the genetic stability in human beings of the attenuated polioviruses now being considered as candidates for a live poliovirus vaccine to be taken orally.

SMALLPOX VACCINE POTENCY ASSAY BY TISSUE CULTURE TECHNIQUE

Tissue culture methods may provide a more satisfactory method for the assay of potency of smallpox vaccine than the rabbit titration method. For many years, the routine method of measuring the potency of smallpox vaccines has been the inoculation of smallpox vaccine dilutions onto the skin of the rabbit. Considerable natural

resistance and variable sensitivity are encountered among rabbits in the performance of this potency test, and the test itself depends on the skill of the operator. Investigators have been searching for a testing system which would permit more precise standardization of the vaccine.

Dr. Ernest Cutchins, DBS, and his co-workers, have studied the sensitivity of a number of primary and continuous cell cultures to infection by vaccinia virus. Of the various cells treated, primary monkey kidney and primary rabbit kidney cells were found to be most susceptible to infection. Primary rabbit kidney cells were chosen as the test system for vaccine potency assay because of general availability and economy.

Trypsinized kidney cells from adult rabbits are grown in roller tubes. Serial 10-fold dilutions of the vaccine to be tested are then inoculated into the tubes. For a period of 7 days, the cell cultures are observed at intervals for the presence of virus infection. The virus content of the test vaccine is calculated from the number of rabbit kidney tubes which become infected. The titer of the test vaccine is compared with that of the control vaccine similarly treated.

No evidence of variation in susceptibility from lot to lot of rabbit kidney cells has been observed. The experiments suggest that titration in tissue culture provides a sensitive and reproducible technique for the measurement of the viral content of smallpox vaccines. DBS scientists and manufacturers of smallpox vaccine are presently conducting studies to determine the correlation of this method of potency testing with the method routinely used.

SKIM MILK UTILIZED FOR CELL CULTURE MAINTENANCE

DBS scientists have discovered that autoclaved skim milk provides a nutritional environment in which continuous cell lines can be sustained in a viable condition fully sensitive to virus effects. The new medium's nutritive value is attributed to the milk proteins which are believed to be responsible for the maintenance of cell lines for almost a month with supplemental feedings at weekly intervals.

Animal serum is the essential constituent of most maintenance media for continuous cell cultures, but many such sera have been found to contain "inhibitors" to a wide range of viruses. Skim milk is free of most of the virus inhibitors found in animal serum. It has a potential application as a standard medium for the safety testing of virus vaccines and for the comparative assay of a variety of viruses

on a number of different cell cultures. It can also be used in the overlay media for virus plaque assays. DBS scientists are presently using the new medium to test the sensitivity of live cells to poliovirus, adenovirus, and Coxsackie viruses.

Two contributions to basic research were byproducts of the skim milk medium. The observation was made that the skim milk lost its opalescence when fed to monkey kidney cells. Ultraviolet absorption techniques revealed that the clearing of the milk was caused by the dissolution of the protein casein and the consequent release of amino acids. The causative factor of this phenomenon proved to be a protein-splitting enzyme which was also shown to break down gelatin and serum albumin.

Other cell cultures, using skim milk as a medium, produced an enzyme activator in animal and human sera. This activator is distinguished from other cell-released substances by its specificity for those enzymes in blood which dissolve clots. Both the enzyme and the activator are non-dialyzable and are stable to freezing and thawing. The enzyme, however, can be passed through ultrafine glass filters; the activator cannot.

Various cell lines are being studied to determine whether they produce either of these two substances and if so, how much they produce. Other studies are in progress to determine the effect of infection by poliovirus on the production of these substances by the tissue cells. Such information would be of potential value in the identification of cell lines and as an index for virus infection of cells.

MOUSE TUMOR AGENT HAS VIRUS PROPERTIES

Two years ago, Dr. Stewart, NCI, and Dr. Eddy, DBS, reported their discovery of a mouse tumor agent that is carried in tissue culture which appeared to have biological and physical properties of a virus. The agent has since been found to produce multiple tumors in randomly bred and inbred strains of mice, Syrian hamsters, rabbits, and rats, if the animals are inoculated at a very young age.

As a result of further collaborative work this year, using techniques that are routinely applied to identify viruses, Drs. Eddy and Stewart gave further support to the interpretation that one virus entity, separable from the cell, is responsible for the malignant growths induced in mice and hamsters. Because of the occurrence of multiple neoplasms which differ histologically in both the hamster and the mouse (Eddy, Stewart, Young, and Mider, J. Natl Cancer Institute), there was a question as to whether one or more viruses were involved

as etiological agents. Present data indicates that the neoplasms are caused by only one virus, now known as the SE polyoma virus. It has been shown that virus-containing fluids in ultimate dilutions, filtered through 120-micromilliliter pore-size membranes, heated at 60° C for 30 minutes, lyophilized, stored at -70° or 20° C, treated with glycerol, ether, or trypsin, or absorbed on red cells, induce tumors in hamsters which are similar to those induced by undiluted or untreated virus-containing tissue culture fluid. If several viruses are involved, they are apparently similar in all the above characteristics.

Further evidence that the virus is a single entity has been obtained by the recovery of the virus from plaques. The plaques were prepared in stoppered bottle cultures by a modification of the methods of Dulbecco and of Hsiung and Melnick. One milliliter of virus diluted to give an estimated 1-to-100 plaque-forming unit was introduced into each bottle. After two hours incubation, the cell sheet was covered with agar overlay and again incubated at 36° C. On the 16th day, small patches of degenerated cells were observed under the microscope. These cell plaques were added to mouse embryo cell cultures with fluid medium and, when injected in hamsters, caused tumors of the same variety and appearance as those seen when the polyoma virus was propagated in cell cultures with fluid nutrients.

Further findings are: 1) Induction of tumors in hamsters with the agent passes serially in thirty or more monolayer cell cultures; 2) the passage of the virus from mouse to mouse or hamster, from hamster to hamster or mouse, and from mouse to rat to hamster, with intervening tissue culture passage; 3) the induction of a renal tumor in a rat, and tumors of the ovaries, heart, kidneys, and subcutaneous tissues in a hamster with fluid from a parotid gland tumor in a hybrid mouse; and 4) concentration of the polyoma virus may be accomplished by three methods that are used for concentration of other viruses, namely, adsorption on erythrocytes, centrifugation, and alcohol precipitation.

PHOTODYNAMIC METHOD OF INACTIVATING VIRUSES UNDER STUDY

The inactivation of viruses by irradiation with visible light in the presence of a photosensitizing dye is being investigated as a potential method in the preparation of vaccines. Although the photodynamic action of a dye on protozoa was known as early as 1898, when Raab reported that paramecia were killed when exposed to visible light in the presence of methylene blue dye, the technique has not been widely applied.

The possible use of this phenomenon in methods of preparing vaccines prompted Drs. C. W. Hiatt and Jerome J. Helprin, DBS, to study the

inactivating effect of ordinary visible light on various viruses in the presence of trace amounts of toluidine blue dye. Bacteriophage, a virus which attacks bacteria, was used as a model in the particular study because of the high concentration of virus obtainable and the precision of its assay.

A small amount of toluidine blue dye was mixed with a suspension of bacteriophage and exposed in a thin layer to light from an incandescent bulb. The dye absorbs the light energy at a specific wave length and, since the target point of this energy is apparently the nucleic acid of the virus, the antigenic protein of the virus remains unaffected although the reproductivity of the virus is destroyed. In studies using two different phages, it has been demonstrated that there is a wide differential in rate of uptake and tenacity of binding of the photosensitizing dye.

This selective method of inactivation, if it could be applied to other viruses, would 1) obviate some of the physical limitations of current inactivation methods in which the margin between inactivation of the virus and destruction of its antigenic properties is narrow; 2) be of potential use in the classification of viruses, since some appear to be susceptible to this system and others do not; and 3) contribute to a better understanding of the structure of virus particles.

NEW ANTIBODY FOR HUMAN BLOOD FACTOR DISCOVERED

A rare anti-k blood serum has been discovered in DBS Blood and Blood Products laboratories. Its unique ability to react directly with red blood cells to detect the cellano (k) factor makes it a potentially valuable diagnostic tool that can be used without application of indirect typing methods involving Coomb's serums, a prerequisite in the use of almost all other known anti-Cellano serums. (Reported in Vox Sanguinis.)

The newly discovered anti-k reacts as a complete saline agglutinin with specificity at 37°, 22°, and 4° C. The donor source of this serum has maintained the same high antibody level for the past 21 months, thus permitting the accumulation of sufficient serum to supply research workers in other laboratories in this country as well as abroad. It is also being stored in DBS laboratories to provide a dried control standard.

The serum was discovered during the course of genetic studies carried out in collaboration with NIDR, NINDB, and NIAMD. Under this program, several hundred families have been investigated for the frequency of rare blood factors and the detection of atypical blood group antibodies. Such work has been extremely useful in demonstrating the transmission of heritable characteristics and their relation to the ecology of disease.

HIGHLIGHTS OF PROGRESS

OF RESEARCH IN

GENERAL MEDICAL AND BIOLOGICAL SCIENCES

1958

Items of Interest on Research Studies Supported by the Division of General Medical Sciences

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Of paramount importance in the creation of the new Division of General Medical Sciences was the need to strengthen support of research in the sciences basic to medicine. This reorganization is manifested in terms of the program responsibilities given to the General Research Grants Branch of the new Division.

Fundamental research supported by the Division continues to provide the stimulus and necessary clues for break-throughs in knowledge on which most progress in clinical medicine depends. Within the broad scope of scientific inquiry supported by this activity are such basic fields as genetics, cell biology, the biogenesis of polynucleotides and nucleic acids and the proteins, growth and development, experimental embryology and teratology, fertility, fetal physiology, and endocrine physiology.

In addition to these preclinical interests, the Division also supports research in many health and medical problems of an applied nature that do not fall within the special interests of any Institute.

Among the special research areas of non-categorical interest, environmental health deserves urgent attention because of the pressing problems associated with water and air pollution control, radiation, food contamination, and occupational health. Another special area of interest is accident prevention, especially in view of the high mortality due to accidents in the ages up to 35. The field of experimental anesthesiology, a comparatively neglected research area, is also sponsored by the Division in view of its growing importance and the need for more knowledge of body functions under the influence of the various anesthetics.

Some examples of the research accomplishments that have stemmed from the Division's program are herewith presented.

Prepared, January 1959

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ISOTOPE "TRACERS" AID PROTEIN SYNTHESIS STUDIES

By tracing the metabolic pathway of isotopically labeled amino acids in bacteria, DGMS grantees have discovered new clues as to how these substances become the "building-blocks" in protein.

A new approach to the important problem of how cells synthesize proteins is discussed in Proceedings of the National Academy of Sciences. By feeding isotopically labeled amino acids to bacteria, Drs. P. D. Boyer and M. P. Stulberg, University of Minnesota, were able to deduce important information about the pathway of incorporation of amino acid into protein by measurement of what happens to oxygen atoms that are lost from amino acids when protein is made.

Their studies showed that when bacteria incorporate amino acids into protein, oxygen 18 (a "heavy" non-radioactive isotope of oxygen), which is coincidentally lost from the amino acid carboxyl group, appears almost entirely in the lactic acid formed. This proves that the process does not involve elimination of water molecules. The results give strong evidence that, in the synthesis of protein by living cells, one oxygen atom from the amino acid carboxyl group appears first in a phosphate compound, and from there is transferred by a sequence of known reactions to lactic acid. This means that amino acid incorporated into protein passes through an intermediate phosphorylated compound. Such findings give support to the probability that amino acid "activation" systems studied outside the living cell are actually operative in living cells.

The studies also showed (1) a remarkable localization of this process is involved within parts of the living cell, (2) that transfer of amino acids across cell membranes involves little or no oxygen loss, and (3) that a little water (if any) is formed, and resynthesis of cellular protein occurs. The studies were made with bacterial cells for technical reasons, but it is likely that similar reactions occur in animal cells.

NEW CHEMICAL TEST FOR MEASURING WATER POLLUTION

At Kenyon College, Gambier, Ohio, a DGMS grantee developed a colorimetric procedure to provide a more accurate determination of nitrate ions in river water and sewage analysis. The new test is a major step in the search for improved criteria for chemical detection of water pollution.

Previous spectrophotometric methods have varied widely in sensitivity and accuracy, and all too often have been exceedingly time-consuming. The new colorimetric method, as described by Dr. James M. Pappenhagen in the Journal of Analytical Chemistry, is eminently satisfactory for samples containing from 0.01 to 0.1 mg. of nitrate nitrogen. While the technique is suitable for the determination of nitrate ions in river water and treated sewage effluent samples, it is not presently applicable for raw sewage samples.

This single study is part of a concerted research effort to detect and ultimately prevent water pollution, and to insure safe water supplies.

FETAL ELECTROCARDIOGRAPHS AID OBSTETRICAL DIAGNOSES

Fetal electrocardiography has demonstrated its value as a diagnostic and research tool in obstetrics and allied fields. In the American Heart Journal, Dr. S. D. Larks--a DGMS grantee at the University of California, Los Angeles--reported that electrocardiograms can reveal the presence of a living embryo as early as 11½ weeks after conception.

During pregnancy, variations in the tracings give evidence of heart abnormalities in the fetus. The fetal recordings also permit the determination of multiple births as early as 16 weeks after conception. According to the investigator, the tracings can give advance warning of approaching abnormal deliveries.

In addition to its value in obstetrics, the fetal electrocardiogram can provide useful knowledge of early cardiac development, and help bridge the gap between embryology and obstetrics.

TRANSITION OF AMINO ACIDS TO PROTEINS, OTHER MACROMOLECULES

DGMS grantees have postulated the steps involved in the transition of amino acids to proteins and nucleic acids. Their present studies, together with other known experimental evidence, are helping to provide clues as to the origin, structure, and biosynthesis of these large molecules.

DGMS grantees, Sidney Fox, Allen Begotsky, Kaoru Harada, and Peter D. Hoagland of Florida State University, are providing

evidence that suggests a "Darwinian evolution" at the molecular level. Their work is directed toward the search for a "primordial" protein and the mechanism of its production.

As reported in the Annals of the New York Academy of Sciences, the studies are designed to simulate spontaneous generation of proteins and nucleic acids. By simulating the terrestrial temperatures believed to exist at the dawn of life, the investigators were able to produce a variety of amino acids--the building blocks of protein--from a few selected biochemical staples. Their findings reveal a pattern of formation that parallels the biochemical changes that take place in the "building-up" processes of the living cell. This suggests a possible pathway from non-living matter to the living cell. The presence of ureidosuccinic acid in the thermal reaction products suggests to biologists a possible mechanism for "inheritance" or perpetuation of these reactions. This can be inferred because ureidosuccinic acid is a component of nucleic acids which are known to be the substances that transmit the physical characteristics and metabolic pathways of living organisms.

The broad biological significance of this research may be seen in the hypothesis that once spontaneous generation of protein is launched and provided with a mechanism of repetition, subsequent development may be subject to environmental factors which lead to directional changes in a manner analogous to Darwinian evolution.

SYNTHETIC SMOG REDUCES MOUSE REPRODUCTION RATE

DGMS investigators have shown that smog, synthetically reproduced by reacting gasoline and ozone, significantly reduces the ability of mice to conceive. The finding is a significant outcome of experimental and clinical studies aimed at assessing the effect of atmospheric pollutants on health.

The possibility that air contaminants may have an adverse biological effect at the low concentrations which regularly occur in urban areas has been a matter of serious public health concern. The present study is among the many being conducted in the Los Angeles area where the problem is aggravated by geographic, meteorologic, and industrial factors which favor high pollutant concentration.

The DGMS supported study was initiated to select and evaluate a biological system capable of responding to low concentrations of air contaminants. In addition to finding that synthetic smog impairs the reproductive ability of mice, the investigation determined that the effect of this

smog was especially severe on newborn mice, practically all of which died prior to weaning.

Scientists Paul Kotin and Marilyn Thomas reported these findings in the AMA Archives of Industrial Health.

PLASTER OF PARIS FOUND USEFUL IN BONE GRAFTS

DGMS grantees have demonstrated that large quantities of plaster of Paris can be added safely to bone grafts used to fill defects in bone. This finding stems from preliminary studies by one of the grantees, Dr. Leonard F. Peltier, who used plaster of Paris to fill defects in the radial bone of dogs. It was found that the plaster was slowly absorbed from the area of implantation, and that when the membranous tissues surrounding bone were present, regeneration of bone occurred in some of the animals.

Dr. Peltier and his associate, Dr. Duane Orn, have shown more recently that the addition of plaster of Paris to bone grafts taken from the bone of the same dog does not appear either to promote or inhibit the survival of the graft, or the regeneration of the bone. The addition of the plaster to bone grafts from other dogs (homogenous grafts) resulted in a small increase in the number of defects showing full regeneration. While this small increase may not be significant, it does draw attention to the possibility that the "take" of homogenous grafts may be influenced favorably or unfavorably by additives--a prospect which deserves further attention.

The most important finding of this investigation, which is described in Surgical Forum, is that the reaction of the tissue to the implantation of large quantities of plaster of Paris in no way interferes with the normal development of bone.

ROLE OF ALCOHOL IN ACUTE PANCREATITIS STUDIED

Preliminary tests on animals have clarified the role of alcohol in the development of acute pancreatitis, a disorder commonly associated with alcoholism.

Although acute pancreatitis has been observed to follow ingestion of large amounts of alcohol, little is known of alcohol's action in producing this disorder. In a series of experiments on dogs, DGMS investigators Antonio Boba, Arthur A. Stein, Yoshihiko Nakamura and Samuel R.

Powers, Jr., of the Albany Medical College of Union University, have shown that alcohol causes excessive gastric secretion and emesis. Gastric acid stimulates pancreatic secretion, and vomiting may force bile from the duodenum into the pancreas. The ensuing pancreatitis, it is suggested, results from the activation of protein-splitting enzymes in the actively secreting pancreas, and this activation is due to the reflux of bile in the pancreatic duct.

In the tests, described in Surgical Forum, the scientists demonstrated that pancreatitis (in dogs) will develop only if gastric acid and bile are present simultaneously in the duodenum. The severity of the inflammation appears to be related to the amount of the gastric acid and the presence or absence of vomiting. In cases where a common channel for pancreatic and biliary secretions had been artifically created and bile could readily pass into the pancreas, an increased flow of acid into the duodenum intensified the inflammation.

On the basis of these studies, it seems that alcoholism may be a stimulating or potentiating factor in the genesis of spontaneous clinical pancreatitis. As in dogs, alcohol would seem to act in two ways: first, by stimulating the production of gastric acid, which on contact with the duodenal mucosa results in increased secretory activity of the pancreas; and second, by inducing vomiting which forces some bile from the duodenum into the pancreatic ducts.

PANCREAS BELIEVED INVOLVED IN CREATINE SYNTHESIS

Evidence has been presented which suggests that the pancreas, in addition to its well known role in carbohydrate and protein metabolism, may play an important role in the biosynthesis of creatine, a compound present in certain animal tissues, particularly muscle. Creatine is believed important in energy production.

The likelihood of this additional pancreatic function is reported by Dr. James B. Walker, a DGMS grantee at Baylor University College of Medicine, Houston, Texas, in a paper appearing in the Proceedings of the Society for Experimental Biology and Medicine. Dr. Walker has demonstrated for the first time the presence of a high concentration of a transamidinase in mammalian pancreas.

Transamidinase is one of the two enzymes known to be involved in the biosynthesis of creatine from its amino acid precursors. Until Dr. Walker's finding, it was

believed to be present in the greatest concentrations in the kidney. Other organs contain this enzyme, but in concentrations thought to be too weak to be of physiological significance.

TRANQUILIZER PRODUCES PSEUDOPREGNANCY IN RATS

Preliminary experiments have revealed that one of the newly introduced tranquilizers is capable of inducing pseudopregnancy in rats.

In a study supported by the Division of General Medical Sciences and Lederle Laboratories, Dr. Joseph T. Velardo of Yale University School of Medicine observed that administration of the drug, 1-(2-hydroxyethyl)-4[3-chloro-10-(phenothiazinyl)-propyl]-piperazine, allows corpora lutea (ovarian endocrine bodies that help maintain a normal environment necessary for fetal development) to remain functional and thus capable of maintaining a state of pseudopregnancy.

The author believes that the tranquilizer acts upon specific areas in the brain (i.e. the hypothalamus) that are responsible for the release of a hormone which maintains the functional capacity of the corpus luteum. If these findings are demonstrated in humans, they may have the following implications. A delay in the regression of the corpus luteum, resulting in retardation of the subsequent ovulation, would postpone the period when fertilization could take place. On the other hand, a compound capable of extending the functional state of the corpus luteum may find therapeutic use in women whose pregnancy often terminates in its early stages because of the inability of the corpus luteum to maintain its functional state.

Dr. Velardo's experiment, reported in Fertility and Sterility, extends and confirms the findings of other investigators who have induced pseudopregnancy in animals by reserpine and chlorpromazine, other commonly used tranquilizers. The clinical import of this research is further reflected in the wide-scale use of tranquilizers and the possible role of these pharmacologic agents on the hormonal control of various complex physiological functions.

HUMAN MOVEMENT PATTERNS STUDIED BY PHOTOGRAPHS

DGMS grantees have developed a photographic method for the time-motion analysis of human movements. The use of color-coded, multiple-image photography

in a study of the reflexes involved in sitting, standing and walking is helping the DGMS investigators to elucidate the psychophysiologic principles underlying human posture.

Reporting this study in the Journal of Psychology, Drs. Frank P. Jones, D. N. O'Connel and J. A. Hanson of the Institute for Applied Experimental Psychology, Tufts University, found that many human movements follow typical patterns of response. These were determined by recording both the stimulus and the response by means of stroboscopic photography which traces the direction of a movement by a sequence of changing colors.

The use of color is a definite advantage over the black and white pictures taken earlier by the DGMS investigators and others. For example, an uplifted arm that is permitted to fall, inevitably rebounds slightly, producing confusing images in the serial photographs. In color, however, such a pattern can be easily interpreted since the direction of movement is indicated by a sequence of changing colors.

It is believed that this method, because of its accuracy and convenience, will shed light on the neuromuscular mechanisms and anatomic relations involved in the postural movements of the head, neck and trunk.

TOXIC EFFECTS OF INSECTICIDE ON HUMANS INVESTIGATED

The toxic effects of a new organic agricultural insecticide were studied in rats to permit a comparison of its toxicity with that of other chemically related insecticides. The need for this type of research is particularly critical because of the wide-spread use of these "systemic" insecticides on growing crops, and the possibility of residues in unprocessed foods.

DGMS grantees, Thomas J. Bombinski and Kenneth P. Dubois, have evaluated both the acute and subacute toxicity of the new compound which is chemically related to various other commercially used agricultural insecticides. The results of this study, reported in the AMA Archives of Industrial Health, permit a comparison of the toxicity of this compound (O, O-diethyl S-2-ethyl-2 mercaptoethyl phosphorothiolate) with that of other phosphorus-containing cholinergic insecticides.

Symptoms caused by acutely toxic doses of the new compound, typical of those produced by cholinergic organic phosphates, indicate that the compound gains access to both the central

and the peripheral nervous systems. Tests on rats demonstrated that atropine affords a considerable measure of protection against levels of poisoning that might be encountered in accidental exposure.

In view of the possibility that subacute poisoning in humans could result from repeated exposure during application of the insecticide, the DGMS grantees also tested the compound in rats for its cumulative toxic effects. The results of these tests indicate that subacute poisoning requires repeated exposure to relatively high fractions of the lethal dose. Since pronounced symptoms occur during the first few days of exposure to doses of this magnitude, it is mandatory that further exposure be avoided to prevent fatal poisoning in man.

NEW METHOD EVALUATES TOXICITIES OF X-RAY CONTRAST AGENTS

A new method has been developed to evaluate the toxicities of certain dyes used as contrast agents to delineate cerebral arteries by X-ray. The method, reported in the Journal of Neurosurgery, employs the spinal column of the dog as a testing site because its spinal column corresponds closely in structure and blood supply to that of man. This study, originally supported by the Division of General Medical Sciences, is now receiving co-equal support through NINDB. The grantees--Drs. G. Margolis, G. T. Tindall, R. L. Phillips, P. D. Kenan, and K. S. Grimson of Duke University School of Medicine--reported that neurologic deficits and anatomic lesions in the spinal cord--two of the several indicators used to evaluate toxic action in this study--are alterations that are permanent in character and subject to more accurate quantitative analysis than those used as current standards of toxicity.

It remains to be seen whether these relative toxicities can be correlated with their effectiveness as contrast agents. Studies such as these should help to permit the selection of the optimal agent for use in this clinical procedure.

PROTEIN PATTERN IN DRIED BLOOD CAN IDENTIFY OWNER

By means of paper electrophoresis, DGMS grantees have demonstrated real differences in the protein pattern of dried blood of various individuals. This has been shown to be of immediate practical value in crime detection since it is not always possible to identify all known blood groups in dried blood.

Paper electrophoresis is a method for the separation of charged molecules in an electric field. Larger molecules move at a slower rate and therefore travel a shorter distance than smaller molecules. The pattern of this movement is measured on paper and serves to identify the various proteins.

In preliminary tests, described by Paul L. Kirk and Charlotte L. Brown of the University of California in the Journal of Forensic Medicine, samples of fresh finger blood, drawn from laboratory personnel, were deposited on slides which were dated, dried, and stored. It appears that periods approaching one year's standing of a dry stain have little influence on the outcome of the tests. Longer intervals of time, in some instances, weaken the pattern.

Several samples of dry blood actually involved in criminal cases were also tested. Blood from a murder victim was clearly and readily distinguished from blood of the victim's spouse. Blood of another murder victim was tested and compared with the dried blood of the convicted murderer which had been obtained at the scene of the crime. The differences between the two were sufficiently marked to prevent confusion of the two persons involved and aided in the identification of the criminal.

STUDIES IN PREGNANCY WASTAGE

Endocrinological experiments on rats have shown that injections of adrenocorticotropic hormone (ACTH) reduce litter size and produce a large number of stillbirths. This was demonstrated by Dr. Joseph T. Velardo, a DGMS grantee at Yale University School of Medicine who administered ACTH on the day of mating, and on six subsequent days. The study is one of many DGMS supported research projects aimed to elucidate: (1) the complex role that endocrine factors play in the physiology of reproduction, and (2) the causal factors leading to pregnancy wastage.

Dr. Velardo's work, reported in a recent issue of the American Journal of Physiology, supports the contention that numerous interactions exist among the hormones of the adrenal cortex and the ovaries. Furthermore, it would appear that a precise ratio must exist between hormones of the adrenal cortex and those of the ovaries to bring about successful gestation in laboratory animals. It is hoped that future investigation will clarify the mechanisms involved in adrenal-gonadal interrelationships, and provide important clues to the many-faceted problems of pregnancy wastage.

ALLERGY CAN CAUSE SKIN GRANULOMAS

Two dermatologists, Drs. Walter B. Shelley and Harry J. Hurley of the University of Pennsylvania, have demonstrated both experimentally and clinically that granulomas (tumor-like cell masses) can be caused by an allergy. Their work was prompted by the finding that deodorants containing the metallic element zirconium caused granulomas to form on the skin.

Granuloma formation, which consists of closely-packed specialized skin cells, are considered one of the significant reaction patterns of the body in disease. They are involved in the basic body changes in such chronic diseases as tuberculosis, leprosy, and syphilis.

In this DGMS-supported study reported in Nature, healthy volunteers were given injections of an aqueous solution of sodium zirconium lactate in and under the skin to build up sensitivity. Six months later, one of the test subjects suddenly developed large masses at the site of all the early injections. The reaction was found to be due to a state of allergic hypersensitivity to zirconium.

Additional proof of the allergic basis of the granulomas came from tests on six patients who had developed the masses from zirconium deodorants. They were injected with diluate solutions of the zirconium compound, and in all cases granulomas formed at the site of injection. Control subjects showed neither clinical nor histological response to these injections.

The authors believe that further research should tell which of the granulomas found in disease are allergic and which are non-allergic. It should be noted that this work has added significance because many of the recently marketed poison ivy medications contain zirconium.

FURTHER FRACTIONATION OF PITUITARY GLAND HORMONE

Drs. Choh Hao Li and Phil G. Squire of the University of California have found in the course of their work with interstitial-cell stimulating hormone (ICSH) that more than one protein component in an extract from sheep pituitary glands possesses ICSH activity. Interstitial cells are a non-germinal component of the testis. They are known to secrete the male hormone that gives rise to secondary sex characteristics and behavior.

The investigators' report in the journal, Science, is devoted to a biophysical study of one of these components, β-ICSH. Its interstitial-cell stimulating activity was estimated by its ability to increase the weight of the ventral prostate in rats from which the pituitary gland had been removed.

DGMS-supported studies of this kind are adding significant knowledge to the understanding of some of the specific factors involved in sterility in man, and may lead to an improved therapy for certain testicular deficiencies.

NEW THEORY EXPLAINS COMPOSITION, OSMOTIC STRENGTH OF GASTRIC JUICE

DGMS grantees bolster evidence for new theory about the stomach's production of hydrochloric acid. This theory postulates that both electrical and chemical forces are involved in the production of the constituents of gastric juice.

In earlier studies, Dr. Warren S. Rehm of the University of Louisville, Kentucky, developed a theory about the gastric production of hydrochloric acid. This stated that; (1) there is "active transport" (i.e., by chemical means and not by physical forces such as simple diffusion) of the negative chloride ion in the stomach, (2) this transporting mechanism gives rise to electromotive forces, and (3) the flow of current thus created controls the liberation of hydrogen ions. In the present study reported in the American Journal of Physiology, the DGMS grantee and his co-workers, Warren H. Dennis and William A. Brodsky, amplify this theory and attempt to fit it to known facts about the composition and osmotic strength of gastric juice.

The theoretical explanation further developed in this study is that the "active transport" mechanism previously mentioned is in operation and, in addition, other ionic movements occur due to electrochemical potential gradients. This means that chloride ions are released into the gastric juice by active transport in the parietal cells; this sets up current flow between this site and the surface epithelial cells where there is liberation of hydrogen ions at the latter site. Due to the resultant electrochemical potential gradient, some sodium and potassium ions are also liberated and some chloride ions are reabsorbed at the surface epithelial cells. Thus is explained the presence of sodium and potassium in the gastric juice. The decrease in osmotic strength is related to the loss of chloride, and to the fact that water moves with the sodium and potassium ions.

There are a number of theories on the mechanism of gastric acid secretion and many variants of each of these. The solution of this problem is of importance in understanding the secretory process in general and, more practically, the normal and abnormal functioning of the stomach. Dr. Rehm's theory is of interest in that it helps explain the basic mechanism and some of the variations in gastric juice composition which are normally encountered. Studies such as this may lead to improved treatment for peptic ulcer and other abnormal gastric conditions.

EVIDENCE OF RELATIONSHIP BETWEEN PINEAL GLAND AND THE GONADS

Studies of melanin pigmentation in the pineal gland (whose function is unknown) have produced evidence suggesting that this gland is interrelated to the gonad--the sex gland for reproduction.

DGMS grantee Enrique Santamarine, Rutgers University, studied the incidence of melanin--pigmentation produced by metabolic activity--in 880 pineal glands of cattle. Reporting his findings in the Canadian Journal of Biochemistry and Physiology, Dr. Santamarine observed that in pregnant cows over five years of age, melanin was present in a few glands; non-pregnant cows of the same age exhibited the pigmentation in slightly increased numbers of pineal glands.

No melanin was found in the glands of heifers 18 months of age, nor in non-castrated bulls four and five years of age. In castrated bulls between 18 and 24 months of age, however, melanin was present in 49.6 percent of their pineal glands. It was also noted that castration caused hypertrophy of the pineal gland.

Concludes the investigator, "Whatever the intimate mechanism of melanin formation in castrated bovine pineal gland, this phenomenon seems to give strong evidence of a relationship between the pineal gland and the gonads."

NEW SYMPTOM COMPLEX FOUND IN INFANTS

DGMS investigators report a new syndrome observed in infants with symptoms of edema, pallor, and marked irritability. Extensive studies suggest that the symptom complex is related to an abnormality in protein metabolism at the cellular level.

Investigations by Drs. Robert A. Ulstrom, Nathan J. Smith, Ernest M. Heimlich, and Kazuko Nakamura at the University of California reveal that the symptom complex is due to an abnormal decrease in the amount of blood protein and a transitory anemia. Follow-up tests with radioactive amino acids indicate the low protein level in blood (hypoproteinemia) resulted from a relative failure to utilize amino acids in addition to a rapid degradation of plasma proteins. A study of the dietary intakes preceding and during the development of the anemia and hypoproteinemia shows that the disturbance is not related to a deficiency of any essential protein component in the diet of the afflicted children.

The underlying cause of this disorder remains unknown at the present time. It is the investigators' opinion, however, that the syndrome is the result of an inborn error in protein metabolism. Further investigations of the molecular structure of the serum proteins, as well as of the factors influencing protein metabolism, are recommended to establish the basic reason for the rapid degradation rate of proteins in these children.

The studies were reported in the AMA Journal of Diseases of Children.

INSULIN-PRODUCING CELLS TRANSPLANTED SUCCESSFULLY

DGMS grantees have transplanted successfully the insulin-producing cells of the pancreas to other sites within the same animal.

The study, described in Surgical Forum, was conducted by Drs. William R. Rundles and Henry Swan at the University of Colorado School of Medicine, Denver. In a series of staged operations, the pancreas of the dog was transplanted into an artificial "pocket" within the rectus muscle. This enabled the insulin-producing islet cells to continue functioning but eliminated the acinar tissue which had prevented previous transplantation of pancreatic tissue because of the autolytic effect of the pancreatic digestive enzymes.

The transplanted tissue was then regrafted to a new location on the abdominal wall and showed evidence of active function after a latent period of from 30 to 52 days. Removal of the autograft, after a period of survival without insulin, produced a diabetic state indicating that the graft had been the sole source of insulin.

Of the eighteen dogs originally utilized in these studies, only four dogs survived the various stages of the transplantation. Additional trials will be needed to substantiate the success of these experiments.

RADIOISOTOPES DETECT BACTERIA IN DRINKING WATER

A method utilizing radioisotope techniques has been developed to permit rapid detection of coliform organisms. The method is likely to be of further use in other fields where bacteriological identification, culturing, or control is important, such as the milk and food industries, the sterilization industry, bacteriological warfare countermeasures, and medical diagnostic laboratories.

The time now required to complete a bacteriological analysis of drinking water by current standard methods is approximately 48-72 hours. Consequently, one can only know what the quality of the water was rather than what it is.

The method developed by Gilbert V. Levin, Venton R. Harrison, and Walter C. Hess of Georgetown School of Medicine, Washington, D. C., detects the presence of small numbers of coliform bacteria within several hours. (The sanitary index of drinking water quality is the coliform organism which is universally present in sewage or contamination therefrom.)

The DGMS grantees' test, reported at the 2nd United Nations International Conference on the Peaceful Uses of Atomic Energy held in Geneva September 1-13, is a refinement of the standard method for the identification of coliform organisms. The standard test for detecting these organisms requires sufficient cell multiplication to yield direct visual evidence of colonies or gas production (carbon dioxide). The gas is produced by the action of the organisms on the sugar, lactose, contained within the culture medium. By labeling the lactose with a radioactive isotope (C^{14}), the scientists are able to detect and measure by means of a specially adapted Geiger counter exceedingly small amounts of carbon dioxide gas. This, in turn, reveals the presence of small numbers of coliform bacteria that are not readily detectable by any previously used method.

FLUID LOSS IMPLICATED AS FACTOR IN RADIATION DEATHS

Recent studies of the factors that bring on rapid death following heavy radiation of the small bowel have shown

that infection is not a major cause of death as previously believed. In a search to determine the nature of these specific factors, DGMS grantees at the University of Mississippi, Jackson, have undertaken preliminary studies of the fluid and electrolyte alterations following irradiation of a segment of small bowel.

Drs. M. Don Turner, Robert D. Sloan, James C. Griffin, Jr., and Tom D. Norman report in Surgical Forum that irradiation of the distal 50 to 60 percent portion of the small bowel of dogs produced a moderate to severe diarrhea resulting in marked dehydration. By measuring total body water before and after the radiation, the investigators demonstrated that large amounts of sodium and chloride were lost from the extracellular fluid. Their work suggests that large amounts of sodium, potassium, and chloride also accompanied the intracellular water loss.

Although more studies of the acid-base picture will have to be carried out, the investigators believe that sufficient data are on hand to implicate fluid loss as a major cause of death in animals subjected to heavy bowel irradiation.

ULTRASONIC TEST EXPLAINS ENERGY TRANSMISSION INTO LIVING CELLS

A new concept of how supersonic waves can transmit vibration energy into living cells is suggested by biophysical experiments of DGMS grantees. The study is relevant to the interests of current medical researchers, since supersonic vibration techniques are put to use by diagnosticians and physical therapists.

A new mechanism that demonstrates flow patterns and stresses produced by ultrasonic waves in fluid provides an explanation of how these waves can transmit vibration energy into living cells.

In this mechanism devised by DGMS grantees, Francis J. Jackson and Wesley L. Nyborg of Brown University, Providence, Rhode Island, a film which forms the lower boundary of a liquid-filled cell is set into oscillatory motion by contact with a vibrating metal cone which produces supersonic waves. The oscillatory motion, in turn, produces vibrations throughout the immediately surrounding fluid which are traced photographically by the use of indicator particles.

The study which is reported in the Journal of the Acoustical Society of America supports the contention that the phenomenon known as cavitation may be responsible for the transmission of supersonic vibrational energy into the cell. Supersonic waves may strike a cell directly, and this transmits vibrational energy into the cell. However, many cells escape this direct impingement, due to a number of factors, one of which is the dissipation of the vibrational energy through the formation of bubbles (cavitation) in the areas adjacent to the cells. It is these bubbles, according to the DGMS grantees, which, by touching the cell wall, transmit a good deal of vibrational energy into the cell.

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Items of Interest on Research Studies

Conducted by the Division of Research Services

In recent years, progress in medical research has been accelerated as a result of extensive collaboration between scientists in widely different disciplines. Today's medical scientist, in order to make maximum progress, also works closely with teams of highly-skilled professional and technical personnel, trained and equipped to provide a variety of technical, scientific, and engineering services. At the National Institutes of Health, these services are furnished by the staff of the Division of Research Services.

The Division comprises over 800 persons. The group includes instrument makers, biometricians, engineers, architects, veterinarians, librarians and translators, scientific illustrators, medical photographers, and editors and writers, as well as animal caretakers and others who patiently perform a variety of unglamorous but essential tasks.

Working side by side with Institute investigators, DRS scientists and technologists often collaborate on problems that involve both the research under way and the service to that research. For instance, precision instruments, germ-free or specially bred animals, culture media, and other research tools, often not available from commercial sources, are constantly developed and improved by the DRS staff.

Supplemental studies to improve the quality and efficiency of existing services, such as equipment sterilization and animal surgery techniques, are also frequently undertaken.

To maintain the quality level of its services, the Division occasionally anticipates needs and initiates studies over and above those specifically requested. Recent projects include studies of NIH's distilled water system and development of an operating room air control system.

Prepared, January 1959

In many instances, collaborative or individual research conducted by DRS personnel results in a scientific publication. Often, newly developed research tools and techniques are shared through consultations with other medical institutions or commercial manufacturers.

STUDIES LINK SMOKING, CANCER MORTALITY, AND CORONARY DISEASE

This year, Dr. Harold F. Dorn, Chief of the Biometrics Branch, DRS, reported the results of a two-and-one-half-year statistical study, conducted jointly with the National Cancer Institute, establishing a relationship between the use of tobacco and death from lung cancer and coronary disease.

The report presents data indicating that the death rate from lung cancer for regular cigarette smokers is 10 times greater, and the rate from all causes 58 percent greater, than for nonsmokers. It shows that regular cigarette smokers who stop smoking have a lower mortality rate than those who continue to smoke. However, reducing the number of cigarettes smoked per day, but continuing to smoke regularly, does not result in a reduction in the death rate.

The death rate from coronary heart disease among regular smokers of cigarettes is 63 percent higher than the rate for nonsmokers, Dr. Dorn's report indicates. It also suggests that cigarette smokers have a higher mortality rate than do nonsmokers from cardiovascular and respiratory disorders, from ulcers of the stomach and duodenum, and from cirrhosis of the liver.

These conclusions are based on mortality statistics of nearly 200,000 government life insurance policyholders, most of them veterans of World War I, who were queried on their smoking habits. As life insurance policies were terminated, the Biometrics Branch was notified by the Veterans Administration. If termination was due to death, information was collected from physicians and hospitals concerning the cause of death and method of establishing the diagnosis.

The study group also provided information on other factors--for example, occupation and residence--that are being investigated for their relationships to smoking and mortality. (This study is also reported in Highlights of Cancer Research.)

ANIMAL SURGERY TECHNIQUES AID RESEARCH STUDIES

New surgical techniques for complete removal of the pineal and pituitary bodies in the dog have been developed by the Animal Hospital Section, Laboratory Aids Branch, DRS, for scientific studies in the National Heart Institute.

An adjustable loop of surgical wire threaded through a hypodermic needle is being used successfully to excise the tiny pineal body, often "lost" in suction tube removals. A suction needle, insulated with polyethelene tubing, has been developed to grasp the stalk of the pituitary body so that it may be cauterized, thus insuring complete hypophysectomy without damaging other brain areas.

These techniques assist scientists in determining more about the function of the pineal and pituitary bodies under certain experimental conditions.

IMPROVED HEART PUMP NOW IN USE AT NIH

Technologists in the Instrument Section, Laboratory Aids Branch, collaborating with cardiacsurgeons of the National Heart Institute, have perfected a more efficient, electrically controlled "artificial heart" pump, now used routinely in cardiac operations at NIH.

Considerably smaller and more compact than previous pumps, the device does not damage blood cells--a disadvantage common in previous designs. Incorporated in the pump is a dual power system for use in the event of power failure. The pump can also be operated manually. Other improvements include disposable sterile connective tubing, enclosed tubing at pressure areas, and a clear plastic window to insure full vision for the operator.

Incorporated into the heart pump are electrically variable stroke controls that maintain a constant blood volume at any desired rate. The pump produces up to seven liters of blood per minute--three more than required by the average human heart.

An electric "venous reservoir," recently developed by the Instrument Section, has been perfected for incorporation into the heart pump. Using electrodes, this device controls the blood level in the oxygenator and the blood temperature.

The new pump is noiseless, easily constructed, and requires little maintenance.

Although specifications have not been published, the design has been released to other medical institutions and to several commercial manufacturers.

CONTRAST MEDIUMS USED IN ANIMAL SURGERY

A tool for evaluating the results of animal surgery without sacrificing experimental animals is being applied by veterinarians in the Animal Hospital Section, Laboratory Aids Branch, DRS.

The introduction of iodinated organic compounds into selected body cavities makes it possible to obtain clear radiographs of surgical results and anatomical variations. Now performed routinely, the technique eliminates time-consuming autopsies or surgical examinations formerly required to evaluate certain types of surgery.

MINIATURE ELECTRODES FABRICATED FOR BRAIN STIMULATION STUDIES

Miniature electrodes, smaller in diameter than a human hair, are being produced in the Instrument Section, Laboratory Aids Branch, for use in brain stimulation studies in NIMH and NINDB.

Mechanically injected into the brain, these minute devices transmit electrical impulses to an electroencephalograph, which records the activity within brain tissues. Recorded patterns enable investigators to locate brain areas that are affected by certain diseases and drugs.

Watchmakers, working with the aid of jewelers' glasses and microscopes, painstakingly fabricate these microelectrodes by hand. Instruments devised to facilitate the process include a miniature lathe used in winding pure silver wire onto the electrode. Precise fabrication has resulted in microelectrodes acclaimed by scientists using them as "surpassing any known to date."

Some 20 other types of micro- and macroelectrodes have been developed and built by Instrument Section craftsmen to meet the needs of NIH scientists. Some are capable of registering millionths of a volt--the electrical potential of a single cell--in millionths of a second.

NEW METHOD OF STERILIZING HEAT-SENSITIVE MATERIALS

Members of the Sanitary Engineering Branch, DRS, have evaluated the effectiveness of new gas-sterilization methods for heat-sensitive instruments, equipment, and other materials used in NIH laboratory and clinical studies.

A commercially-developed aerosol-type container utilizing ethylene oxide--a known bactericide and fungicide--and a non-flammable gas has proved safe and efficient in extensive tests of microbicidal effectiveness and mechanical reliability.

Perishable materials that can be sterilized by this method range from infected books to plastic components of the NIH heart-lung machine.

MICROMANIPULATOR CONTROLS PLACEMENT OF ELECTRODES

A new device to control the movement of microelectrodes used in studying the central nervous system has been developed by the Instrument Section, Laboratory Aids Branch, DRS.

Known as a micromanipulator, the instrument is of such accuracy that microelectrodes can be inserted into the human brain or spinal cord from angles of penetration adjustable to divisions of one micron, or .000039 of an inch. The device also permits scientists to reproduce this angle at any time after the initial experiment.

The extreme accuracy of this instrument has created universal interest in the field of micromanipulation.

NEW STRAINS OF EXOTIC ANIMALS NOW AVAILABLE

Five species of gerbils from Egypt, meriones from Japan, and waltzer guinea pigs are among new types of unusual animals now stocked or raised by the Animal Production Section, Laboratory Aids Branch, DRS, for use in research studies at NIH.

Studies in several NIH Institutes indicate that the new types may be more susceptible to some parasitic diseases than the usual laboratory animals. Colonies of gerbils (small nocturnal rodents) are already proving of value in tropical disease studies. Other animals recently added to the genetically controlled animal colony include chinchillas, two types of hamster mutations, and a species of rat in which jaundice frequently occurs spontaneously.

COMPLEX LABORATORY PROCEDURES AUTOMATIZED

The Instrument Section, Laboratory Aids Branch, DRS, collaborating with NIAMD chemists, has designed and built a completely automatic system for separating and analyzing the components of mixtures. Primarily fabricated for use in analyzing certain hormones in urine, the unit is capable of continuous, unattended operation, thus freeing several technicians for other duties and increasing the laboratory's production.

An ingenious component of the assembly consists of a highly accurate device for the controlled mixing of two liquids at variable rates and volumes. The device solves a classic problem in column chromatography, where different ratios of solvents must be added to solid extracts in the chromatography column, which separates solutes as they pass through at different rates.

Mathematically determined mixing ratios of solvents, plotted on an aluminum template, are translated into a proportionate electrical voltage that controls pump motors mixing the solvents. The entire unit is constructed of glass and Kel-F, a special plastic, thus preventing contamination of the solvents.

Test tubes, passed under the chromatographic column on a moving rack, collect the separated components, or fractions. Each fraction is equally divided for automatic ultraviolet or chemical analysis, and results are recorded on a moving graph. A portion of each fraction is then mechanically transferred to a storage area for further use.

A wide variety of mixture, even the most complex, can be analyzed by the assembly, which is so designed that any component unit is adaptable to a variety of uses.

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